Desert Tortoise Conservation Genetics: Genetic Variability Among and Within Sonoran Populations

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Sonoran populations of the Desert Tortoise (*Gopherus agassizii*) occur in rocky foothills throughout southwestern Arizona and northwestern Mexico. Although tortoise populations appear to be isolated from each other by low desert valleys, radiotelemetry data suggest that tortoises occasionally move long distances between populations. Concern has arisen that habitat fragmentation due to recent human land use changes may hinder the ability of tortoises to move between populations. We used molecular techniques and radiotelemetry to examine movement patterns of desert tortoises in southern Arizona. We collected blood samples from 170 individuals in 8 mountain ranges surrounding Tucson and 1 east of Phoenix. We analyzed mitochondrial DNA sequences and developed 6 novel microsatellite markers useful for conservation genetic studies of this species. Two microsatellite loci exhibited low variability (2-3 alleles), but four were highly variable (8-27 alleles). Genetic differentiation among Sonoran populations was low ($F_{ST} = 0.037$, $p < 0.001$). We distinguished the pattern of gene flow from that of recent ancestry by testing for reductions in population size (bottlenecks) using both the methods of Garza and Williamson (2001) and Cornuet and Luikart (1996). Gene flow estimates between populations suggest that populations exchanged individuals historically at a rate greater than one migrant per generation (private alleles method; Nm = 5.5). There was a positive correlation (r = 0.554, $p = 0.030$) between genetic and geographic distance of population pairs; a pattern characteristic of isolation by distance. During the study, we observed a radiotelemetered tortoise move more than 32 km to another mountain range and back and we documented the anthropogenic barriers it encountered. Desert tortoises are capable of and sometimes motivated to disperse great distances and these movements result in the exchange of genetic material among adjacent populations. Because many historic dispersal routes are no longer available to desert tortoises as a result of anthropogenic landscape change, informed management strategies need to be developed to insure the long-term persistence of Sonoran desert tortoise populations.

INTRODUCTION

Survival of wildlife populations is influenced by both demographic and genetic processes, including the extent of genetic variation (heterozygosity) within populations and the flow of genetic material among them (Soulé 1986). Gene flow in most animals is accomplished through immigration. Therefore, estimates of gene flow can tell us the extent to which populations are connected or have been connected in the past. Restricted movement among local populations, which can result from construction of roads, canals, and other barriers, may lead to increased levels of inbreeding, smaller effective population size, loss of genetic variation and increased risk of extirpation. Understanding the population genetics of a species is necessary to assess long-term stability and to make management recommendations regarding reserve size, necessity for corridors, and translocation strategies.

Sonoran Desert populations of the desert tortoise (*Gopherus agassizii*) generally inhabit areas of rocky foothills associated with saguaro cactus (*Carnegiea gigantea*), foothill paloverde (*Parkinsonia microphylla*) and desert ironwood (*Olneya tesota*). Mountain ranges with these leguminous tree and mixed cactus vegetation communities

are scattered throughout southern Arizona and many contain populations of desert tortoises. Although foothill populations appear to be isolated by low desert valleys, radiotelemetry data have shown that tortoises are capable of making long distance movements between populations (Barrett et al. 1990, Averill-Murray and Klug 2000, Schwalbe et al. 2002). However, the importance of these movements and whether they contribute to gene flow is unknown. Determination of the extent to which these disjunct populations interact is an important aspect of desert tortoise conservation.

The Sonoran Desert "population" of the desert tortoise is not federally listed but is considered a Species of Special Concern by the Arizona Game and Fish Department (AGFD 1996). Although a number of threats to tortoises have been identified, loss of habitat currently represents the greatest threat in rapidly growing communities such as Phoenix and Tucson (AIDTT 1996). In the Tucson area, many thousands of acres of tortoise habitat have been recently lost due to large residential developments in the foothills of the Santa Catalina, Tortolita, Rincon, and Tucson Mountains. Development reduces the size of populations and isolates them with barriers such as highways and canals. There is a strong management need to identify the important connections between tortoise populations before the opportunity to preserve them is gone. Due to recent advances in molecular biology, we are now able to address the issue of connectivity with a greater depth of understanding than previously possible. Because major human development is fairly recent in respect to the generation time of the desert tortoise (life-span probably exceeds 40 years; Germano 1992), the genetic structure of tortoise populations has not likely yet been adversely affected by landscape changes. By measuring gene flow among populations, we obtain a snapshot of the movement patterns of desert tortoises prior to habitat fragmentation. The degree of relatedness of tortoises both within and among mountain ranges has implications for how sustainable small populations may be as they become isolated.

In our study, we examined the genetic relationships of tortoises in eight populations in the vicinity of Tucson and one population northeast of Phoenix. By comparing genetic distance (variation between populations) with geographic distance and calculating migration rates among these populations, we estimated historic rates of gene flow and assessed the degree of isolation currently caused by human barriers. In addition, we evaluated genetic relatedness among individual desert tortoises within a single population located in the Rincon Mountain District of Saguaro National Park while simultaneously gathering information on movements and home ranges using radiotelemetry. In this population, we compared genetic differences among individuals to geographic distances between them to determine if gene flow within the population is random or is influenced by behavior and habitat features such as ridges and drainages.

In this study, we examined two types of genetic markers suitable for population level studies. First, we sequenced a portion of tortoise mitochondrial DNA (mtDNA). DNA sequencing identifies the actual series of nucleic acids in a given region of the mitochondria; polymorphism (degree of genetic relatedness) is estimated based on individual nucleic acid changes among individuals. Mitochondrial DNA loci have been used in previous studies of the desert tortoise (Lamb et al. 1989, Lamb and Lydeard 1994, Ostentoski and Lamb 1995, Britten et al. 1997, McLuckie et al. 1999). However, these prior studies primarily investigated phylogenetic relationships of distinct populations and/or species, and compared relatively few individuals across large geographic areas.

In addition to mtDNA sequencing, we identified and analyzed microsatellite DNA markers. Microsatellites have recently become very popular genetic markers for determining population structure and revealing differentiation among populations (Bruford and Wayne 1993). Microsatellites allow extremely high resolution for the differentiation of individuals and populations, even within small geographic areas such as the Tucson Basin. Microsatellites, or short tandem repeats (STRs), are non-coding repetitive DNA sequences composed of a variable number of tandem repeating motifs. Microsatellites are bi-parentally inherited, considered selectively neutral, and are codominant, thereby allowing both alleles at a locus to be identified in heterozygotes. Microsatellite loci had not been previously used in studies of the desert tortoise, so we identified markers by constructing a microsatellite-enriched genomic library using methods adapted from Hamilton et al. (1999). By identifying variable microsatellites in the desert tortoise genome suitable for population genetic studies, these markers can contribute to future studies of Sonoran desert tortoise, as well as the possible application to the Mojave Desert population of the desert tortoise and other congeners in the United States and Mexico, at least two of which (Bolson and gopher tortoise; *G. flavomarginatus* and *G. polyphemus*) are species of concern.

Objectives

Our Objectives are to:

1. Sequence a region of mtDNA and identify and score informative microsatellite markers within the Sonoran desert tortoise genome.

2. Estimate rates of gene flow and compare genetic distance with geographic distance among several tortoise populations in the Tucson area and a more distant population in Maricopa County.

3. Examine genetic relatedness of individuals within a single population of desert tortoises and assess the role that habitat characteristics and behavior play in shaping distribution of genetic variability.

4. Map potential anthropogenic barriers to tortoise movement among the mountain ranges of the study area.

5. Utilize genetic and spatial information to assess long-term viability of isolated populations of desert tortoises.

6. Make recommendations for management of tortoise populations based on genetic and geographic information.

Hypotheses

We tested the null hypotheses that:

1. There are no significant genetic differences between pairs of desert tortoise populations from adjacent mountain ranges (i.e., that gene flow occurs, or occurred until recently, between adjacent mountain ranges).

2. For non-adjacent pairs, genetic distance is correlated with geographic distance.

3. Within a single population, genetic variation between individuals is random and not structured (i.e., not associated with behavior or habitat characteristics).

METHODS

Study Sites

We sampled desert tortoises from eight sites in Pima and Pinal counties in the vicinity of Tucson and from one population northeast of Phoenix in Maricopa County. Between 8 and 38 tortoises were sampled from each population, depending on population size, for a total of 170 tortoises (Table 1, Figure 1). In the Tucson Basin, we sampled tortoises from the Picacho, Silver Bell (Ragged Top) and West Silver Bell Mountains, as well as from small populations at Florence Military Reservation, Desert Peak and Tumamoc Hill. We also sampled populations from both the Tucson Mountain and Rincon Mountain districts of Saguaro National Park (SNP). In Maricopa County, we collected samples from the U.S. Forest Service/AGFD monitoring plot in the Mazatzal Mountains (Sugarloaf).

Table 1. Number of samples collected from nine desert tortoise populations in southern Arizona.

POPULATION	**# of samples**
Desert Peak	12
Florence	8
Picacho Mountains	18
Ragged Top	22
Rincon Mountains (SNP)	38
Sugarloaf	27
Tumamoc Hill	9
Tucson Mountains	18
West Silver Bell Mountains	18
TOTAL	**170**

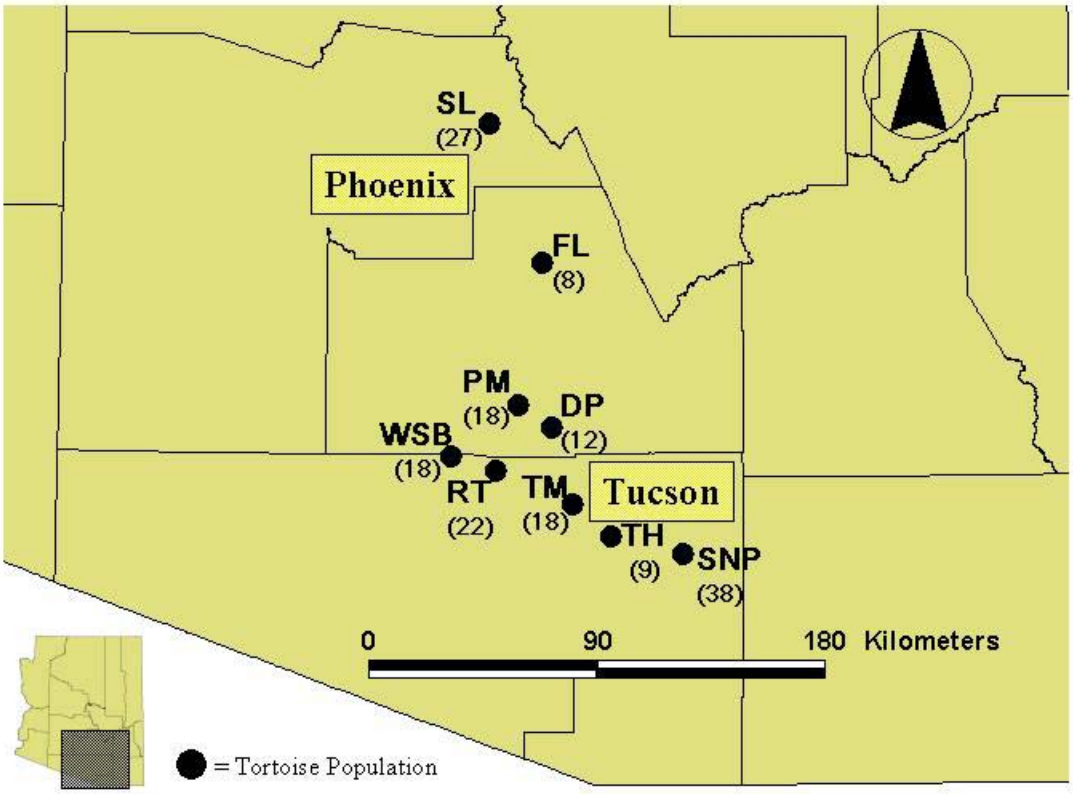

Figure 1. Location of and number of tortoises sampled from nine desert tortoise populations in southern Arizona; Desert Peak (DP), Florence Military Reservation (FL), Picacho Mountains (PM), Ragged Top (RT), Rincon Mountains (Saguaro National Park; SNP), Sugarloaf (SL), Tumamoc Hill (TH), Tucson Mountains (TM), and West Silver Bell Mountains (WSB).

Field Methods

We hand-captured and processed tortoises March-October, 2000 and 2001 using standard methods (e.g., Murray and Schwalbe 1997), following Arizona Interagency Desert Tortoise Team guidelines (Averill-Murray 2000). We processed tortoises at the site of capture. Occasionally, tortoises were carried from capture sites to a central field processing location and returned to the point of capture within 4 hours. We transferred tortoises by placing them in a clean, cloth bag that was moistened to maintain humidity and temperature in between processing and during transportation. Bags were cleaned and disinfected before reuse.

Unmarked tortoises were marked using established notching systems for desert tortoises (Ernst et al. 1974, Averill-Murray 2000), with new numbers following those from previous studies at each site (Appendix A). We also marked tortoises with a small, epoxy-covered number on the fourth vertebral scute for easy identification if recaptured. At sites where there was not an established numbering system or where the numbering system was unknown, we only affixed an epoxy label to tortoises to prevent resampling. We used standard procedures for processing tortoises (Murray and Schwalbe 1997, Averill-Murray 2000). We measured midline carapace length (MCL) of captured

tortoises using calipers and a metric tape measure and used a Pesola spring scale to measure mass. We recorded age, sex, and health parameters, including shell anomalies, disease symptoms, and presence of parasites. We took precautions to not transfer potential disease between individuals by wearing latex gloves and washing equipment and hands with the veterinary disinfectant chlorhexidine diacetate (Nolvasan; American Home Products Corporation). Tortoise handling protocols were approved by the University of Arizona (IACUC 00-084).

We collected blood by brachial venipuncture. We placed tortoises on a pedestal (such as an empty coffee can) to immobilize them during processing. Samples were collected in a calm and quiet manor to minimize stress to the animal. We sanitized the topical area using an alcohol swab. We collected less than 1cc of blood with a syringe and 25⅝-gage needle and stored it on ice with an EDTA buffer (lavender-topped tube; Becton-Dickinson). On occasion, we collaborated with AGFD personnel who were collecting blood samples for disease studies. During these times, blood was collected by jugular venipuncture and buffered in lithium heparin (green-topped tube; Becton-Dickinson).

To alleviate problems of dehydration, we rehydrated tortoises handled during vulnerable times of year (spring and late summer) and those that voided a substantial volume of urine. Tortoises were rehydrated with 2-4% body weight of saline solution (0.9% NaCl) at approximately the body temperature of the tortoise. We injected fluid through a 22-gage needle at the junction of the left forelimb and plastron, approximately 2 cm into the body cavity, between the plastron and pleuroperitoneum (coelomic cavity).

Molecular Techniques

All genetic procedures and analyses were conducted at the Genomic Analysis and Technology Core (GATC) at the University of Arizona.

DNA Isolation

Tortoise blood samples collected in the field were stored at 4 °C in lithium heparin or EDTA-buffered Microtainers (Becton-Dickinson). We isolated total DNA by overnight lysis with proteinase K at 55 °C, followed by a standard phenol/chloroform extraction and isopropanol/sodium acetate precipitation (Sambrook et al. 1989). We resuspended the DNA in low TE (10mMTris-pH 8.0, .01mM EDTA) and used a FLx 800 fluorescence reader (Bio-Tek Instruments, Inc.) to measure its concentration. Working stocks were diluted to 5 ng/µl.

Mitochondrial Amplification

We initially examined genetic diversity using mitochondrial DNA (mtDNA) sequences in a subset of 38 samples distributed across 8 populations. We amplified approximately 987 base pairs of the ND3/ND4 region of the tortoise mitochondria using the primers Nap2 and New Gly (Arévalo et al. 1994). These primers were used successfully in previous desert tortoise research (Britten et al. 1997). Polymerase chain reaction (PCR) was performed in 50-µl volumes with 10 mM Tris-pH 8.3, 3.5 mM $MgCl_2$, 50 mM KCl, 2 units of Taq Polymerase (Sigma-Aldrich), 0.2 mM of each dNTP, and 20 pmol of each primer. The PCR reactions were cycled in a Mastercycler Gradient (Eppendorf) with a 5-minute 94 °C initial denature, followed by 35 cycles of 1 min at

94°C, 2 min at 50.6 °C, 2 min at 72 °C and a final 6 min incubation at 72 °C. We purified the PCR products using the QIAquick PCR purification kit (Qiagen) and sequenced them at the GATC DNA Sequencing Laboratory at the University of Arizona on an ABI Prism® 3700 DNA Analyzer (PE Biosystems). We used Oligo Primer Analysis Software version 6.68 (Molecular Biology Insights, Inc.) to design internal primers used to obtain sequences for analysis (Nap2INT 5'AGGCGGTCAATAATG CTAATC3' and NewGINT 5'TAATAAAACCAGACAATGAAAAAC3'). We evaluated and aligned mtDNA sequences using Sequence Navigator version 1.0.1 (Applied Biosystems, Inc.).

Microsatellite Development

We prepared a microsatellite-enriched genomic library for tortoises based on the methods of Hamilton et al. (1999). The Hamilton et al. procedure isolates DNA containing tandem repeats by hybridizing fragmented DNA with biotin-labeled repeat oligos. The biotin-labeled oligos are bonded to streptavidin-coated iron beads and a magnet is used to separate DNA hybridized to the oligo from that not complementary to the oligo motif. We digested genomic DNA from a single individual (RK486; Appendix 1) with *Rsa*I (New England BioLabs, Inc.) and ligated SNX linkers onto both the 5' and 3' ends of the digested fragments (Hamilton et al. 1999). We experimented with a variety of repeat motifs (10 repeats each) and oligo combinations, including CAA, CTT, ATC, and AGT. We probed ~100 ng of genomic DNA with a single oligo or combination of oligos (2 pmols of each) in a 100 µl volume of 5X SSC, 0.1% SDS, and 50% formamide. We performed the hybridization by heating the reactions to 95 °C for 15 minutes and then stepping down the temperature one degree per minute to 60 °C (hybridization temperature), where the reaction incubated for one hour. We then added 300 µg of Dynabeads M-280 (pre-washed four times in 10mM Tris [pH 7.5], 1mM EDTA, 1M NaCl; Dynal) and incubated the samples at 43 °C with agitation for a minimum of 5 hours. We washed the beads twice at room temperature with 2X SSC, 0.1% SDS, twice at 45 °C with 1X SSC, 0.1% SDS, and then twice at 65 °C (5 °C above hybridization temperature) in 1X SSC, 0.1% SDS for five minutes each. We used a magnet (MPC-E-1, Dynal) to hold the beads and hybridized DNA in the tube while removing wash solutions. We eluted the DNA from the beads by heating them in low TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) at 95 °C for 10 minutes and took the supernatant. We PCR amplified the eluate using the SNX primer with Vent-exo Polymerase (New England Biolabs) as described in Hamilton et al. (1999) protocol. In an alternative modification of this protocol, we digested the genomic DNA with *Alu*I, *Rsa*I, and *Nhe*I and used an oligo of 10 AGC repeats as a probe. We used a hybridization buffer of 12X SSC, 0.1% SDS, hybridized at 75 °C, and heated the last two washes to 80°C.

We cloned the products of this initial PCR using a TOPO TA cloning kit (Invitrogen). We PCR amplified inserts from individual colonies using T7 and M13R universal primers in 25 µl volumes with 10 mM Tris-pH 8.3, 3.0 mM $MgCl_2$, 50 mM KCl, 1 unit of Taq Polymerase (Sigma-Aldrich), 0.2 mM of each dNTP, and 20 pmol of each primer. We amplified directly from colonies by touching each clone lightly with a sterile toothpick and dipping it briefly into the reaction. We performed the PCR reactions in a PTC-100TM Thermocycler (MJ Research, Inc.) with 10 minute 96 °C initial

denature, followed by 35 cycles of 30 sec at 96 °C, 45 sec at 57 °C, 1.5 min at 72 °C and a final 10-min incubation at 72 °C. We purified the products of this PCR using the QIAquick PCR purification kit (Qiagen) and sequenced them at the University of Arizona GATC sequencing service using standard sequencing protocols for the ABI Prism® 377 DNA Sequencer or ABI Prism® 3700 DNA Analyzer (PE Biosystems). We examined sequences for microsatellites using Sequence Navigator version 1.0.1 (Applied Biosystems, Inc.).

We used Oligo Primer Analysis Software version 6.68 (Molecular Biology Insights, Inc.) to design primers to the flanking regions of the microsatellites. Using these novel primers, we performed PCR in 10 µl reaction volumes containing 0.2 µM of each primer, 10 mM Tris-HCl (pH 8.3), 0.25 mM of each dNTP, 0.4 units of Taq (Sigma-Aldrich), 50 mM KCl, 5 ng of genomic DNA template, and between 2.0 and 3.5 mM $MgCl_2$ depending on the locus (Table 3). PCR was performed using a PTC-100TM Thermocycler (MJ Research, Inc.) with an initial 5-min denaturation at 94 °C followed by 35 cycles of 30 sec at 94 °C, 30 sec at the annealing temperature (Table 3), and 30 sec at 72 °C, followed by a 6-min incubation at 72 °C. Some loci required the addition of formamide to improve PCR product amplification. Loci were amplified using 5' fluorescently labeled forward primers (Invitrogen/ Applied Biosystems, Inc.) and sized using an ABI Prism® 3100 Genetic Analyzer and Genotyper® version 1.0 software (PE Biosystems). For each locus, a set of eight tortoises from across our sampling distribution was initially scored to assess if the marker was polymorphic.

In addition, we tested 11 microsatellite loci identified in several other chelonian species: *Chelonia mydas*, (Cm3, Cm58, Cm72, Cm84), *Caretta caretta* (Cc117, Cc7) *Eretmochelys imbricata*, (Ei8) and *Podocnemis expansa* (PE334, PE519, PE107), (FitzSimmons et al. 1995, FitzSimmons 1998, Sites et al. 1999).

Molecular Analyses

Mitochondrial DNA

We estimated nucleotide diversity and polymorphism using DnaSP 3.53 (Rozas and Rozas 1999). Nucleotide diversity (Pi) is a measure of the amount of genetic variability in a sample. The parameter theta (nucleotide polymorphism) is associated with effective population size (N_e). We tested for selective neutrality using Tajima's D (Tajima 1989), which examines the relationship between Pi and theta and is sensitive to processes that reduce or increase heterozygosity in a population. Under neutrality, D is expected to be zero. A positive value for D indicates an excess of heterozygosity, resulting from processes such as a reduction in population size, population subdivision, or balancing selection. A negative value for D indicates a reduction of heterozygosity that could be the result of an expansion event, positive directional selection, or the presence of weakly deleterious alleles (Tajima 1989). We assessed the structure of observed genetic variation in tortoise populations using AMOVA (analysis of molecular variance) in ARLEQUIN version 2.0 (Schneider et al. 2000). AMOVA can be used to examine the variance in gene frequencies while also taking into account the number of mutations between haplotypes (Excoffier et al. 1992). This approach uses Wright's F-coefficients to determine how genetic variation is partitioned among populations within a region and among individuals within populations (Wright 1951).

Microsatellite DNA

Allele frequencies were calculated for each locus in each population and frequency distributions were examined for unique and private alleles. Private alleles are unique alleles with frequencies ≥ 5%. A high proportion of private alleles can suggest population subdivision (Barton and Slatkin 1986). We used ARLEQUIN version 2.0 (Schneider et al. 2000) to detect significant departure from Hardy-Weinberg equilibrium at each locus. The Hardy-Weinberg law describes the equilibrium state of a locus in a randomly mating diploid population by examining the simple Mendelian relationship between allele frequencies and genotypic frequencies. Evolutionary forces such as mutation, migration, assortative mating and genetic drift can cause deviations from Hardy-Weinberg equilibrium. ARLEQUIN follows the procedure described in Guo and Thompson (1992) using a triangular contingency table and a modified version of the Markov-chain random walk algorithm. We used default parameters in ARLEQUIN for all Markov-chain tests and permutations. We tested for linkage disequilibrium (nonrandom association between loci) among all pairs of loci in the entire sample and within each population using a likelihood-ratio test with an empirical distribution obtained by permutation (Slatkin and Excoffier 1996). The inbreeding coefficient (F_{IS}; Weir and Cockerham 1984) was determined for each locus in each population using GENEPOP version 3.1 (Raymond and Rousset 1995).

We used BOTTLENECK (Piry et al. 1999) to identify recent bottlenecks in each population and in the entire sample. This test is based on the assumption that a bottlenecked population (one that has experienced recent reductions in effective population size) will show an excess of heterozygosity over that expected under mutation-drift equilibrium (Cornuet and Luikart 1996). Natural oscillations in population size are not expected to show this excess because rare alleles and heterozygosity are reduced at a similar rate. Expected heterozygosities were calculated using the infinite alleles modes (IAM), stepwise mutation model, (SMM), and two-phase model (TPM). TPM is generally considered most applicable for microsatellite data in that it accounts for the probability that some mutation events will result in the addition or deletion of several repeat units (Di Rienzo et al. 1994). We assessed allele frequency distributions using three statistical tests for each mutational model; sign test, standardized differences test (Cornuet and Luikart 1996), and a Wilcoxon sign-rank test (Luikart et al. 1998). A mode-shift from the beta distribution expected under mutation-drift equilibrium would indicate a recent bottleneck in the population.

In addition to the methods of Cornuet and Luikart used to recent reductions in population size, we also used the method of Garza and Williamson (2001). This method uses a TPM to examine the ratio of the total number of alleles to the overall range in allele size (M). M can be interpreted as the average percentage of intermediate allelic states in a population and its value will decrease when a population is reduced in size. We calculated M for each population and for the total region and then simulated M (10,000 replicates) based on the allelic frequencies of the sample populations using three parameters: theta ($4N_e\mu$), P_S (percentage of mutations that add or delete only one repeat), and $delta_g$ (mean size of larger mutations). Simulations generated a statistic M_C, which is the critical value at which 95% of the simulations of M in an equilibrium population are greater than M_C. A reduction in population size is suggested when $M < M_C$. We used two models; one recommended by the authors (theta = 10, $P_S = 0.9$ and $delta_g = 3.5$), and

a more conservative model based on microsatellite data sets from 20 natural populations, (Garza and Williamson 2001; theta = 10, $P_S = 0.88$ and $delta_g = 2.8$). A theta value of 10 represents an effective population size of 5000 individuals (with mutation rate $\mu = 5x10^{-4}$). This value is a compromise between underestimating the population size of the entire region, and overestimating the population size in each mountain range.

We inferred population structure using AMOVA in ARLEQUIN (Excoffier et al. 1992). We used Wright's F_{ST} (Wright 1951) to determine how genetic variation was partitioned within the region, among populations, and among individuals within populations. We used FSTAT version 2.9.3.2 (Goudet 1995) to calculate bootstrap estimators for significance of F-statistics. Wright's F_{ST} assumes IAM and is one of the most commonly reported statistics for estimation of population structure; however, it can have drawbacks when the mutation rate is high, such as with microsatellites (Balloux and Lugon-Moulin 2002). As a comparison, we also calculated Slatkin's R_{ST} (Slatkin 1995). Slatkin's R_{ST} is an analogue of F_{ST} assuming a SMM and is thought to reflect more accurately the mutation pattern of micosatellites (Balloux and Lugon-Moulin 2002). However, R_{ST} estimates are often accompanied by high variance and may be outperformed by F_{ST} estimates (Gaggiotti et al. 1999). We calculated genetic distances among populations and individuals using ARLEQUIN using pairwise F_{ST} (Reynolds et al. 1983) and compared it to pairwise R_{ST} estimates (Slatkin 1995). Negative F_{ST}/R_{ST} values were treated as zero. Estimates of the number of migrants exchanged per generation between pairs of populations (2Nm) were calculated using Slatkin's $\hat{M}$ (Slatkin 1991). Estimates of $\hat{M}$ for populations with pairwise F_{ST} values ≤ 0 are considered to have an infinite number of migrants. In addition, we used the private allele method of Barton and Slatkin (1986) to calculate migration rates with GENEPOP version 3.1 (Raymond and Rousset 1995). Populations having less than one migrant per generation (OMPG) are susceptible to differentiation resulting from mutation or genetic drift (Wright 1931). In simulation studies, Barton and Slatkin (1986) show that when the effective number of migrants (Nm) is greater than 1.0, the mean frequency of private alleles [P(1)] across populations is maintained below 0.1.

We used the program NTSYSpc version 2.02h (Applied Biostatistics Inc.) to perform Mantel tests to assess correlation between genetic distances and geographic distances among populations. The Mantel test (Sokal and Rohlf 1981) tests the significance of the correlation between matrices by a permutation procedure. If gene flow has been the cause of genetic similarity among populations and geographic distance between populations affects the dispersal of individuals between populations, then the regression between the two should be significant (Slatkin and Maddison 1990).

Radiotelemetry

We assessed within-population genetic structure at two established study sites in the Rincon Mountain district of Saguaro National Park. The first site (Mother's Day Fire) lies entirely within the park boundary; telemetry data from nine tortoises from this site have been collected since 1997 to determine the response of desert tortoises to fire (Esque et al. 1998). The second site (Rocking K) is approximately 6 km south of the Mother's Day Fire (Figure 2) and is located along the park's south boundary and the adjacent Rocking K Ranch. Twenty-five tortoises have been radiotracked at this site

since July 1999 for reproduction studies and for baseline data on a study to determine the response of tortoises to urban development (Edwards 1999, Schwalbe et al. 2002).

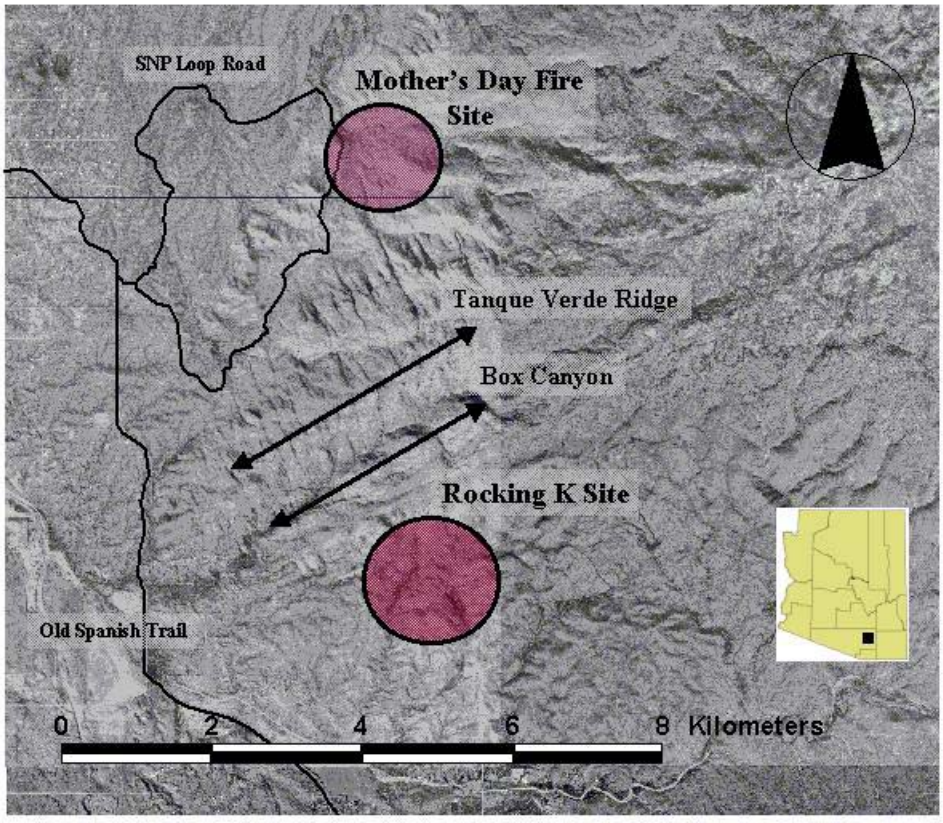

Figure 2. Two study sites within the desert tortoise population at Saguaro National Park (Rincon Mountain District), east of Tucson, Arizona. Geographic features (Box Canyon and Tanque Verde Ridge) potentially separate the sites, but otherwise, the sites are connected by continuous tortoise habitat.

We affixed transmitters (AVM Instrument Co.) to the right front of the carapace using quick-drying epoxy, with the antenna run through rubber tubing to facilitate transmitter replacement (Boarman et al. 1998). Great care was taken to not epoxy across scute seams, where shell growth takes place. Tortoises, on average, were monitored twice weekly during the active season (March-October) and once weekly during winter using a directional antenna and receiver (Telonics Model TR4).

Spatial Analysis

We mapped potential human-constructed barriers to tortoise movement between mountain ranges based on available GIS data. All tortoises sampled for genetic analysis were located to within approximately 5 m using hand-held Global Positioning System (GPS) receivers (GARMIN International Inc.). We calculated the arithmetic mean of all point locations within a population using the Animal Movement Analyst Extension version 1.1 (Hooge and Eichenlaub 1997) in Program ArcView GIS version 3.2 (Environmental Systems Research Institute, Inc.). We used distance between means to determine the geographic distances between populations. We used straight-line distance

as a measure of geographic distance because evidence suggests that long-distance movements of tortoises and other reptiles do not follow natural geographic forms but are essentially linear in nature (Barrett et al. 1990, King and Duvall 1990, Reinert and Rupert 1999).

Within the Saguaro National Park radiotelemetry plots, we estimated tortoise home range size using the minimum convex polygon (MCP) method (White and Garrott 1990) including all point locations for each individual from all years for which telemetry data were available. We compared MCP home ranges between the two sites by multiple regression (Ramsey and Schafer 1997) with explanatory variables of sex, size (MCL), and number of point locations. Multiple regression was analyzed using JMP version 4.0.0 software (Sall and Lehman 1996). We determined the arithmetic mean for each polygon and used that location estimate to determine the geographic distances among home ranges. We used available base coverages from digital orthophoto quarter quads (DOQQs) to map physical features such as ridges and drainages between plots (Figure 2).

RESULTS

Mitochondrial DNA Results

We sequenced 987 base pairs of mtDNA from 38 tortoises distributed across 8 populations (Table 2). We identified five variable mtDNA sites that gave rise to six haplotypes, each of which is characterized by a single base pair difference from the Son01 haplotype (Figure 3). All populations, with the exception of the most geographically distant population (Sugarloaf), shared haplotype Son01 (Table 2). Two individuals in the Picacho Mountains and one individual in the Tucson Mountains had unique haplotypes. The six individuals sequenced from the Sugarloaf population were characterized by two novel haplotypes not shared among other populations. Nucleotide diversity and polymorphism were both very low, (Pi = 0.00045, SD = 0.00045; Theta per site = 0.00121, SD = 0.00065) and Tajima's D was negative, (D = -1.612, $0.10 > p > 0.05$) (Tajima 1989). There was considerable differentiation among populations ($F_{ST} = 0.404$, $p < 0.001$) due to the fact that Sugarloaf does not share haplotypes with the other seven populations. However, when Sugarloaf was removed from the analysis, there was no differentiation detected among populations ($F_{ST} = -0.0773$, $p < 0.001$) indicting that gene flow occurs or has occurred in the recent past. When calculated without the Sugarloaf population, Tajima's D was -1.730 ($0.10 > p > 0.05$).

Table 2. Haplotype distribution of eight Sonoran Desert tortoise populations in southern Arizona.

		SONORAN HAPLOTYPES:					
Population	Number	Son01	Son02	Son03	Son04	Son05	Son06
Desert Peak	2	2					
Florence	4	4					
Picacho Mountains	6	4	1	1			
Ragged Top	4	4					
Rincon Mountains (SNP)	6	6					
Tucson Mountains	6	5			1		
West Silver Bells	4	4					
Sugarloaf	6					1	5
Total	**38**	**29**	**1**	**1**	**1**	**1**	**5**

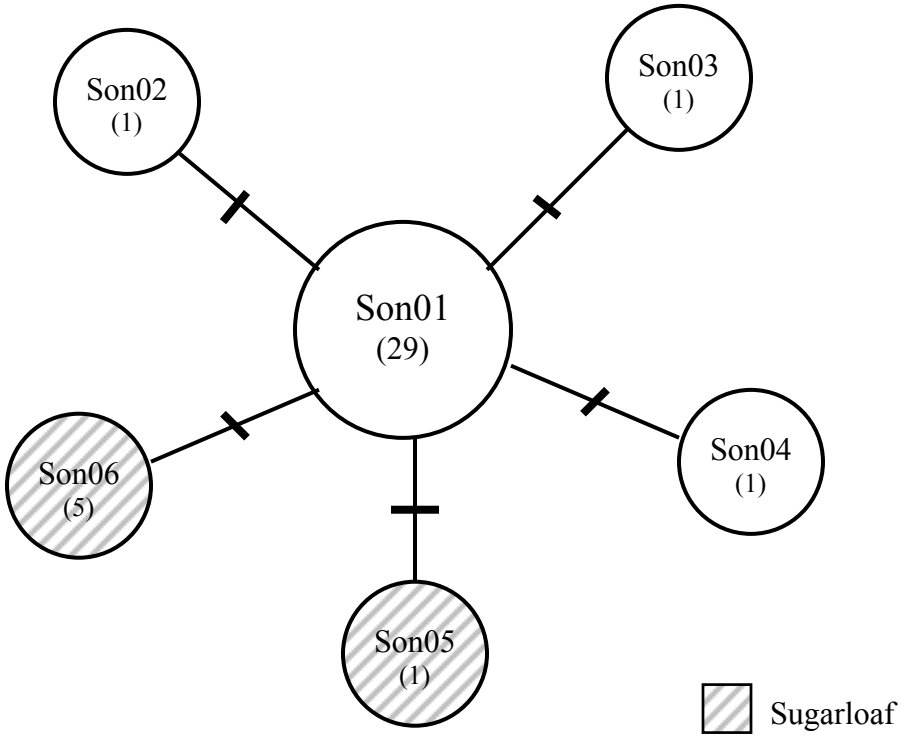

Figure 3. Evolutionary network of Sonoran Desert tortoise haplotypes in southern Arizona. Each haplotype is characterized by a single base pair novel mutation. ( ) = number of individuals exhibiting the haplotype.

Microsatellite DNA Results

Microsatellite Development

The following repeat motifs and combinations of oligos successfully enriched the library for microsatellites: CTT, CAA, and ATC combined, AGT alone, CAA alone, and AGC alone. We amplified five clones derived from the AGC hybridization; two had microsatellites. In six separate hybridization experiments using the CTT, CAA, and ATC oligo combination, we screened a total of 63 clones and recovered eight clones containing microsatellites, four of which were the same locus. From this three-oligo hybridization, we identified the loci *Goag*4 and *Goag*5 (Table 3). We screened 16 clones from the AGT oligo hybridization and found two microsatellite loci, both of which were dinucleotide repeats (*Goag*6, *Goag*7; Table 3). The most effecient hybridization, in regards to the percentage of clones containing microsatellites, was using the CAA probe. (For the CAA oligo, we used a hybridization buffer of 12X SSC, 0.1% SDS, set the step down and incubation temperature to 70 °C, and performed the third wash at 75 °C). Of a total of 150 clones sequenced, 71 contained microsatellites. Of these, many lacked sufficient flanking sequence for primer design and several clones appeared to contain the same microsatellite locus. Only 11 of the clones from the CAA oligo hybridization contained a trinucleotide repeat (*Goag*3). The remaining 60 were dinucleotide repeats in a variety of combinations including the loci, *Goag*8 and *Goag*32 (Table 3).

In total, we identified 53 unique microsatellite loci with sufficient flanking sequence to design primers. We were able to amplify a single amplicon for seven loci. Six loci exhibited variation in our sample set and were used in the present study (Table 3). PCR conditions were optimized for *Goag*5 and *Goag*8 by increasing the 72 °C extension time to 45 seconds and by reducing the DNA to 2 ng for *Goag*6. Both *Goag*5 and *Goag*6 required the addition of 2% formamide to improve PCR product amplification.

In our trial of 11 microsatellite loci from other chelonian species, we successfully amplified two loci in the desert tortoise genome (Cm58, Cc7). Interestingly, the repeat array for both loci were dramatically different in the desert tortoise; for Cm58; $(TA)_5(GA)_3GC(GT)_3$ instead of $(CA)_{13}$, and for Cc7; $(CA)_5(TC)_4$ instead of $(CA)_{14}$. However, without comparing flanking sequences we are not able to confirm if these were in fact the same loci from each species. In a test of eight samples representing eight populations, Cc7 proved monomorphic in our sample of desert tortoises. Cm58 expressed two alleles in our sample set and was used in our analyses (Table 3).

Table 3. Forward (F) and reverse (R) primers, repeat motif, annealing temperature (T_a), and $MgCl_2$ concentrations for PCR amplification of seven variable and two invariant microsatellite loci in the desert tortoise.

Locus	Repeat Motif	Primer sequence (5' → 3')	*Amplicon* Size (bp)	Observed Alleles	PCR T_a (°C)	MgCl (mM)
***Goag*3**	$(CAA)_6$	F: CTG ATT GGT CTG ACT CCC T R: CCT GAT TGC TTC CTG ACA C	375-381	3	61	3.0
***Goag*4**	$(CAA)_{24}$	F: CTC AAC AAA AGG TAA GTG ATG R: GCA TAA AAG TAA ACA GTA AAG TA	110-188	17	57	2.5
***Goag*5**	$(GAT)_{17}$	F: AGG CAA GTG GGT GGT AAT G R: GCG ATT TTG AGG CTT CTT TC	257-365	27	65	3.5
***Goag*6**	$(TC)_8(AC)_{11}$	F: TAA GGG CTA TGA GGA AGA AT R: GTA ATG GTG TGG GTG GGA	360-442	15	53	2.0
***Goag*7**	$(AC)_3(GC)_5(AC)_{11}$	F: TCA ATC CAT TAG TCT TCA CCC R: TTT CTG TTT ATG CTC CGT ATT A	261-281	8	61	3.0
***Goag*8**	$(CA)_{14}TA(CA)_3$	F: ATG CTG ACA ATA GAA CAA GA R: ACA TCT GGG GCT AAA GTG	192	1	57	2.5
***Goag*32**	$(AC)_6$	F: GTG CTG CCT TGA TAA GTA A R: ATA GTT TTC TTT CCT ACA CAT	177-179	2	53	2.5
Cm58	$(TA)_5(GA)_3GC(GT)_3$	F: GCC TGC AGT ACA CTC GGT ATT TAT R: TCA ATG AAA GTG ACA GGA TGT ACC	131-133	2	56.5	3.0
Cc7	$(CA)_5(TC)_4$	F: TGC ATTGCT TGA CCA ATT AGT GAG R: ACA TGT ATA GTT GAG GAG CAA GTG	156	1	59	2.0

Microsatellite Analysis

Microsatellite loci were amplified and sized for all 170 samples from nine populations (Table 1). Allele frequencies for all locus-population combinations are reported in Appendix C. All seven loci were polymorphic in all populations. Loci *Goag*3, *Goag*32, and Cm58 exhibited only marginal variability (2-3 alleles), but loci *Goag*4, *Goag*5, *Goag*6, and *Goag*7 were highly variable (8-27 alleles). These four loci all show gaps in their allelic distributions (Appendix C, Figure C1) and do not appear to follow a strict stepwise mutation model. Unique alleles were found in seven of the nine populations (Table C2). Only four private alleles (frequency $\geq 5\%$) were detected, one in each of four populations. No private alleles had frequencies greater than 7% in a population. The mean frequency of private alleles [P(1)] for our total sample was 0.034.

Three of our loci (*Goag*5, *Goag*6, and *Goag*7) deviated significantly from expected heterozygosities under Hardy-Weinberg proportions using exact probability testing (Table 4). The associated positive inbreeding estimator (F_{IS}) at these loci indicates that these deviations are due to a deficiency of heterozygotes. F_{IS} over all loci for the entire sample was 0.161 (Bootstrapping across loci 99% confidence interval: 0.016 to 0.376). Tests for linkage disequilibrium rejected the null hypothesis of independence of 4 of our 7 loci. However, analyses performed without three of the linked loci (*Goag*5, *Goag*7, and *Goag*32) did not affect the results of the AMOVA, the genetic distance calculations, or the conclusions drawn. We proceeded with analysis using the full set of loci, but also calculated descriptive statistics with the exclusion of the apparently linked loci for comparison. Linkage disequilibrium was still observed within some of the nine populations even when the number of markers was reduced.

Table 4. Hardy-Weinberg proportions and fixation indices at 7 microsatellite loci in the desert tortoise. H_{obs} = observed heterozygosity and H_{exp} = expected heterozygosity. *Prob* = significance level and S.D. = standard deviation of randomization tests for Hardy-Weinberg equilibrium. F_{IS} = Weir and Cockerham's inbreeding estimator (1984).

Locus	H_{obs}	H_{exp}	*prob.*	*S.D.*	F_{IS}	*prob.*
***Goag*3**	0.3626	0.3642	*0.695*	0.0049	-0.008	0.666
***Goag*4**	0.6374	0.6621	*0.510*	0.0022	0.037	0.094
***Goag*5**	0.8830	0.9209	*<0.001*	0.0000	0.041	0.021
***Goag*6**	0.3333	0.6973	*<0.001*	0.0000	0.519	<0.001
***Goag*7**	0.4737	0.6686	*<0.001*	0.0000	0.288	<0.001
***Goag*32**	0.2924	0.2950	*1.000*	0.0000	-0.008	1.000
Cm58	0.2690	0.2873	*0.602*	0.0048	0.048	0.585

We did not find evidence of recent bottlenecks in desert tortoise populations in our study area. When all loci in the sample set were examined together, there was not significant excess or deficiency in heterozygosity (Table 5). The entire sample and each individual population fit the expected beta distribution, suggesting that there have not been recent reductions in population size (Cornuet and Luikart 1996). The method of Garza and Williamson (2001) also did not indicate a recent reduction in population size. All values generated for M (average percentage of intermediate allelic states) fell above the critical value M_C (Table 6).

Table 5. Probability of excess or deficit of heterozygosity across 7 desert tortoise microsatellite loci. Sign test and Wilcoxon sign-rank test (two tails) for mutation-drift equilibrium using three mutation models; infinite alleles modes (IAM), stepwise mutation model, (SMM), and two-phased model (TPM).

	Mutation Model		
	IAM	SSM	TPM
Wilcoxon Test	0.578	0.688	0.078
Sign Test	0.241	0.424	0.164

Table 6. Average percentage of intermediate allelic states (M) for 7 microsatellite loci in nine desert tortoise populations in southern Arizona. Two models were used to generate M_C, the critical value at which 95% of 10,000 simulations of M in an equilibrium population are greater than M_C; one recommended by the authors (theta = 10, $P_S = 0.9$, $delta_g = 3.5$), and a more conservative model (theta = 10, $P_S = 0.88$, $delta_g = 2.8$) based on microsatellite data sets from 20 natural populations, (Garza and Williamson 2001).

Population	**M**	**M_C** (recommended model)	**M_C** (conservative model)
Desert Peak	0.6617	0.5879	0.6345
Florence	0.6631	0.5487	0.5886
Rincon Mountains (SNP)	0.7592	0.6738	0.7297
Picacho Mountains	0.7006	0.6236	0.6718
Ragged Top	0.7218	0.6384	0.6904
Sugarloaf	0.6702	0.6518	0.7070
Tumamoc Hill	0.7189	0.6236	0.6718
Tucson Mountains	0.6108	0.5628	0.6033
West Silver Bell Mountains	0.7226	0.6236	0.6718
Total	0.8113	0.7249	0.7891

Among Population Results

Population Structure

Hierarchical analysis of molecular variance of microsatellite data using IAM revealed that 96.3% ($p < 0.001$) of the observed diversity was in individuals within populations ($F_{IT} = 0.963$), while only 3.7% ($p < 0.001$) of the variation was among populations ($F_{ST} = 0.037$; Bootstraping across loci 99% confidence interval: 0.017 to 0.053). Estimates using SMM also showed very weak differentiation among populations, with 96.8% ($p < 0.004$) of genetic variation in individuals within populations (R_{IT}) and 3.2% ($p < 0.001$) of variation among populations (R_{ST}). F-coeffecients calculated with the exclusion of the potentially linked loci did not differ sufficiently to change the interpretation of the data ($F_{ST} = 0.0355$, $p < 0.001$; calculated for four loci). Wright's F_{ST} and Slatkins's R_{ST} estimates of population differentiation were similar. Estimates of the number of migrants per generation between populations using Slatkin's $\hat{M}$ ranged from 2.9 (Tumamoc Hill/Florence) to "infinite" (Ragged Top/Picacho Mountains) (Table 7). The estimate for effective number of migrants (corrected for population size) between populations using the private alleles method was 5.5 per generation.

Table 7. Slatkin's $\hat{M}$ (absolute number of migrants exchanged per generation between populations) calculated among nine desert tortoise populations in southern Arizona. Estimates of $\hat{M}$ for populations with pairwise F_{ST} values ≤ 0 are considered to have an "infinite" number of migrants.

Population	#	DP	FL	SNP	PM	RT	SL	TH	TM
Desert Peak (DP)	12	-							
Florence (FL)	8	5.0	-						
Rincon Mountains (SNP)	38	16.5	6.0	-					
Picacho Mountains (PM)	18	129.7	7.6	393.3	-				
Ragged Top (RT)	22	16.3	5.0	113.2	Inf.	-			
Sugarloaf (SL)	27	8.4	10.2	7.6	11.3	11.6	-		
Tumamoc Hill (TH)	9	12.9	2.9	18.0	61.9	28.0	4.6	-	
Tucson Mountains (TM)	18	32.0	4.2	22.2	19.0	22.5	6.1	12.4	-
West Silver Bell Mountains	18	18.9	4.4	28.1	151.8	26.2	8.4	17.9	13.7

Table 8. Population pairwise F_{ST} values among nine desert tortoise populations in southern Arizona.

Population	#	DP	FL	SNP	PM	RT	SL	TH	TM
Desert Peak (DP)	12	-							
Florence (FL)	8	*0.091	-						
Rincon Mountains (SNP)	38	0.029	*0.076	-					
Picacho Mountains (PM)	18	0.004	*0.062	0.001	-				
Ragged Top (RT)	22	0.030	*0.090	0.004	0.000	-			
Sugarloaf (SL)	27	*0.056	*0.047	*0.061	*0.042	*0.041	-		
Tumamoc Hill (TH)	9	0.037	*0.148	0.027	0.008	0.018	*0.097	-	
Tucson Mountains (TM)	18	0.015	*0.107	*0.022	*0.026	*0.022	*0.076	*0.039	-
West Silver Bell Mountains	18	0.026	*0.102	0.018	0.003	0.019	*0.056	0.027	*0.035

*indicates significance level P<0.0

Table 9. Geographic distances (in kilometers) among nine desert tortoise populations in southern Arizona.

Population	#	DP	FL	SNP	PM	RT	SL	TH	TM
Desert Peak (DP)	12	-							
Florence (FL)	8	66	-						
Rincon Mountains (SNP)	38	72	128	-					
Picacho Mountains (PM)	18	16	57	88	-				
Ragged Top (RT)	22	27	84	81	27	-			
Sugarloaf (SL)	27	123	59	186	112	138	-		
Tumamoc Hill (TH)	9	48	111	30	63	52	170	-	
Tucson Mountains (TM)	18	31	96	48	44	33	154	19	-
West Silver Bell Mountains	18	41	85	99	33	18	133	70	51

Spatial Analysis

Among the nine Sonoran populations, there was a significant, positive correlation between genetic distance (pairwise F_{ST}; Table 8) and geographic distance (Table 9). The regression accounts for approximately 55% of the variation observed (Figure 4; Mantel test; $p = 0.030$). This correlation was maintained when pairwise R_{ST} was used as a measure of genetic distance ($r = 0.471$, $p = 0.015$).

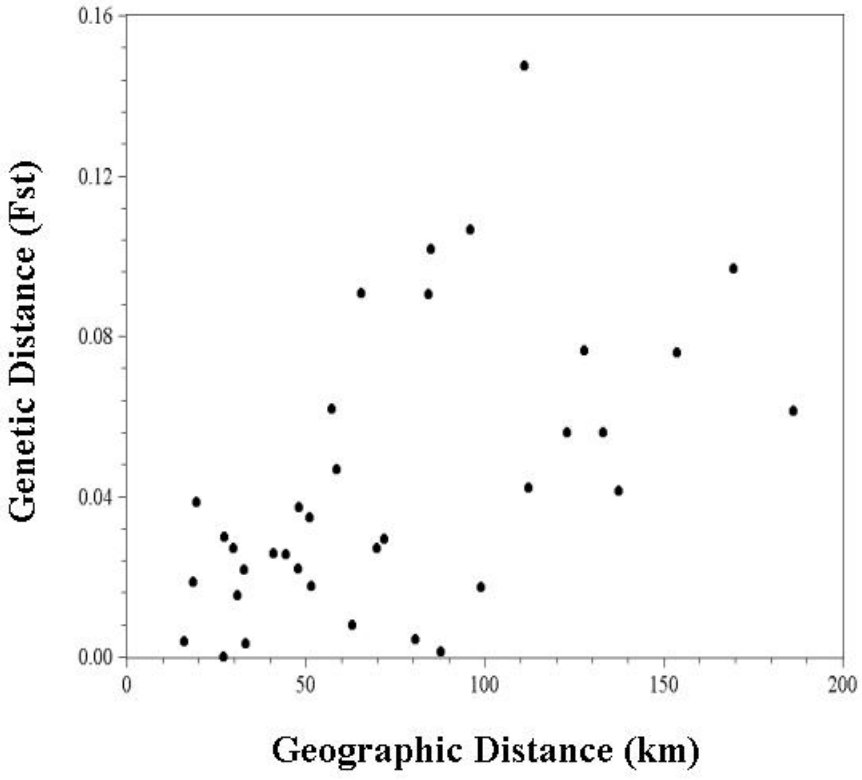

Figure 4. Genetic distance (pairwise F_{ST}) vs. geographic distance (km) among nine desert tortoise populations in the Sonoran Desert. Mantel test ($r = 0.554$, $p = 0.030$)

Movement Barriers

All of the populations we examined have at least a dirt road separating them (Figures 5 and 6). The only population pairs in our sample set that could conceivably still exchange individuals at a natural frequency are Desert Peak/Picacho Mountains and Ragged Top/West Silver Bells. All other connections between populations have human barriers that would seriously hinder or prohibit tortoise movement.

Within Population Results

Radiotelemetry

During the study, no tortoises moved between the Rocking K and the Mother's Day Fire sites at Saguaro National Park. Home range size MCP estimates were calculated for 34 individuals (Appendix B). Home range size for the total sample ranged from 0.33 ha to 81.58 ha ($\bar{x}$ = 18.01 ha, 95% C.I. 11.34 to 24.69 ha). Mean home range at the Rocking K site was 18.54 ha (95% C.I. 9.70 to 27.38 ha, $n = 25$) and mean home range at the Mother's Day Fire site was 16.55 ha (95% C.I. 7.77 to 25.34 ha, $n = 9$). After accounting for explanatory variables of sex, size (MCL), and number of point locations, multiple regression showed no significant difference between the mean home range size between the two sites ($F_{4,33} = 0.425$, $P>0.789$). One individual (RK459) made a long distance movement approximately 32 km out of the Park boundary (Appendix D). Because of this unusual behavior, the MCP home range size for RK459 (10,692 ha) was excluded from home range size comparisons. However, RK459 was included in genetic comparisons between the two sites.

Spatial Analysis

Within the Rincon Mountain population at Saguaro National Park, no population genetic structure was observed between the two radiotelemetry sites despite geographic features that potentially separate them (Figure 2; Tanque Verde Ridge and Box Canyon). We observed 76.9% ($p < 0.001$) of genetic variation within the population, 20.7% ($p < 0.001$) among individuals within the sites, and only 2.4% ($p < 0.056$) between the two sites. Using a SMM, R_{ST} between the two sites was 0.00 ($p < 0.001$). There was no correlation between the genetic relationship among individuals (pairwise F_{ST}) and the geographic distance among their home ranges ($r = -0.072$, $p = 0.289$).

Results of Hypothesis Testing

We are unable to reject the null hypotheses that there are no genetic differences between pairs of desert tortoise populations from adjacent mountain ranges and that for non-adjacent pairs, genetic distance is correlated with geographic distance. Within a single population, genetic variation between individuals was random and not associated with behavior or habitat characteristics.

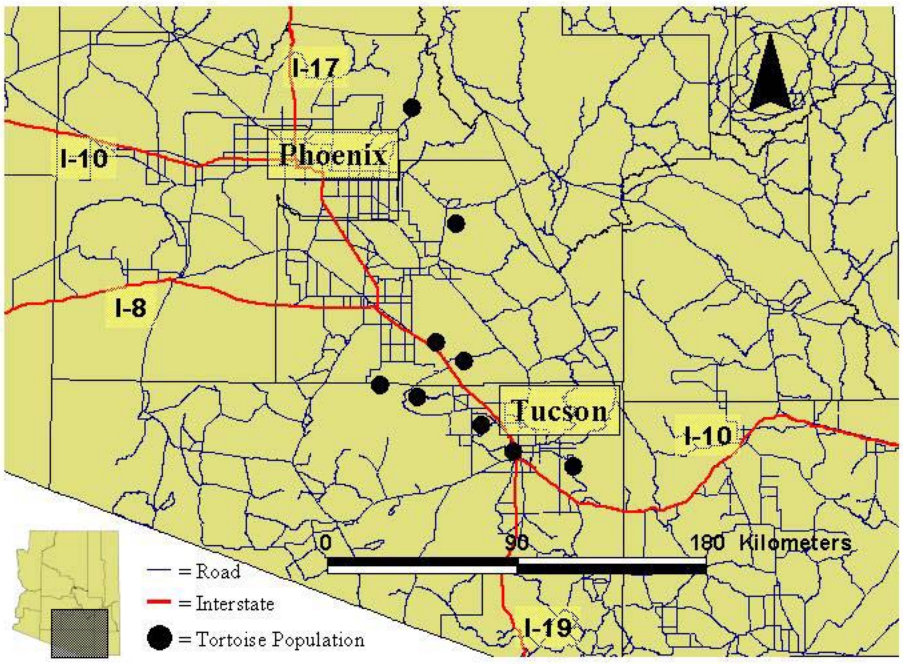

Figure 5. Distribution of interstates and major roads in southern Arizona that hinder or prevent tortoise movement between populations.

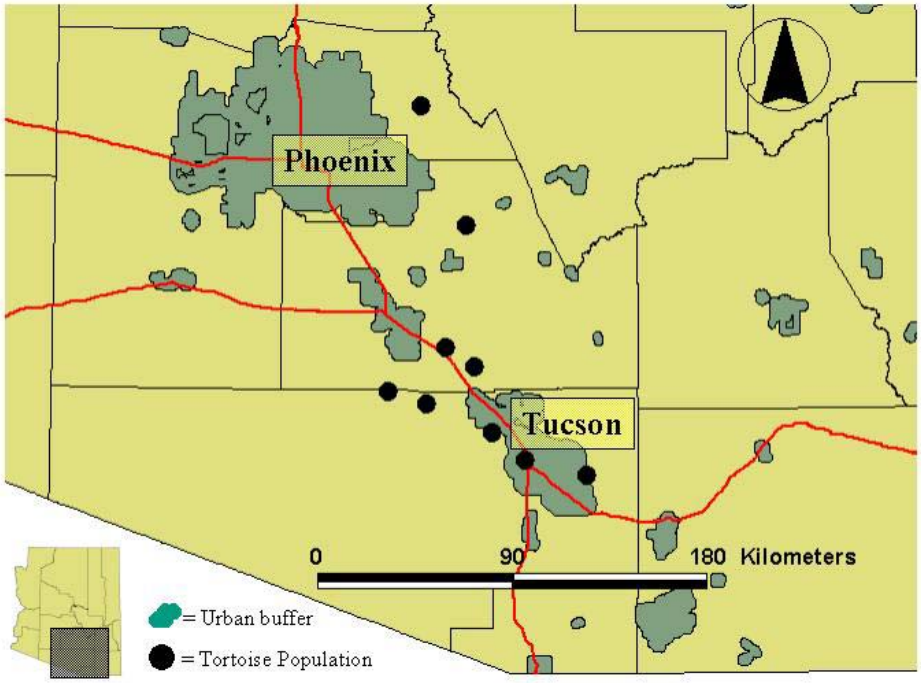

Figure 6. Distribution of urbanized areas in southern Arizona that hinder or prevent tortoise movement between populations.

DISCUSSION

Microsatellite Markers

The departure from Hardy-Weinberg equilibrium and associated positive inbreeding coefficient for some loci in our sample is most likely due to the population structure we observed for desert tortoises. The test for Hardy-Weinberg equilibrium makes a number of assumptions unlikely in natural populations, and observed deviations can be caused by any number of evolutionary forces or stochastic processes, such as migration, mutation, selection, genetic drift, small population size, or overlapping generations. In tortoises, geographic distance is an isolating force that impacts the probability of individuals mating and thus violates the assumption of panmixia. The structured distribution of low frequency and intermediate alleles across populations and isolation by distance we observed in our study (see below) make Hardy-Weinberg an unreasonable expectation for this species. Deviations from Hardy-Weinberg equilibrium can also result from non-amplifying alleles. However, we believe the likelihood of this is small because all samples amplified for at least one allele whereas we would expect some samples to not amplify at all (homozygotes) if null alleles were present in the population.

The test of linkage disequilibrium assumes Hardy-Weinberg proportions, so linkage estimates may be incorrect due to the departure from Hardy-Weinberg equilibrium (Excoffier and Slatkin 1998). A structured population will exhibit allele associations as a consequence of non-random mating that are not a result of linkage within the genome. The possibility that some of our markers exhibit linkage may limit some of the conclusions that can be drawn from this analysis, but did not impact our estimates of gene flow.

Genetic Variability

We assert that the low degree of nucleotide diversity (pi) and polymorphism (theta) among mtDNA sequences is an attribute of desert tortoises maintaining a long-term, small effective population size. Consistent with these findings, in a study of desert tortoise populations in the western and eastern Mojave Desert, Rainboth et al. (1989) came to the conclusion that the low degree of variation observed among allozymes was also a result of small population size. Evidence of small population size in tortoises is supported by census data (Swann et al. 2002). We did not find an excess of heterozygosity or distortion of allelic frequencies in our autosomal loci indicative of a recent (<1000 generations) genetic bottleneck that would otherwise explain a small population size.

The desert tortoise has been present in the Sonoran Desert of Arizona since at least the late Pleistocene (10,250 to 30,000 years ago; Van Devender et al. 1977), and mtDNA analysis allows us to examine population changes in the more distant past. Avise et al. (1992) suggest that desert tortoises have an approximately two-fold slower rate of mtDNA evolution than that observed for mtDNA in birds and mammals. This slower mutation rate of mtDNA relative to that of STRs allows us to ascertain events deeper in history. The negative value calculated for Tajima's D based on mtDNA indicates that there may have been demographic or selective processes that reduced diversity (such as an expansion event) in desert tortoise populations in the more distant past (> 10,000 years ago). However, as it applies to current management implications,

we believe the genetic similarity among tortoise populations revealed by microsatellite analysis is indicative of gene flow and not recent ancestry.

Phylogeography

The phylogeographic pattern of mtDNA haplotypes and rare microsatellite alleles among desert tortoise populations is indicative of intermediate gene flow in a species not subdivided by long-term zoogeographic barriers (Avise et al. 1987). Although the higher degree of divergence observed in mtDNA sequences in the most geographically distant population suggests that Sugarloaf may be isolated from sampled populations in the Tucson Basin, the autosomal data indicate that gene flow does occur to this population but at a lower rate as a function of geographic distance. We suspect that with a larger sample size, shared haplotypes would be found among all populations. The marginal divergence observed in mtDNA sequences, the low frequency of private microsatellite alleles across populations, and the significant correlation between genetic and geographic distance among populations suggests that the genetic relationship among desert tortoise populations is characteristic of isolation-by-distance (IBD; Kimura and Weiss 1964). In a study of tortoises in the northeastern Mojave Desert, Britten et al. (1997) also found evidence consistent with an IBD effect using allozyme and mtDNA data. The desert tortoise is perhaps the ideal organism for the IBD model; one that is distributed across the landscape in isolated patches and for which the difficulty of dispersal is function of geography. Geographic distance separating populations is the major limitation to panmixia of all populations. Within continuous habitat, however, gene flow occurs at random in the population and topographic features do not appear to contribute to within-population genetic structure, as exemplified in our Rincon Mountain population.

Gene Flow

Gene flow occurs, or has occurred until recently, among desert tortoise populations. Historically, tortoises successfully moved between populations and reproduced. The lack of differentiation among populations suggests that dispersal resulting in exchange of genetic material must have occurred historically at a rate of at least one migrant per generation (OMPG) to alleviate differentiation resulting from mutation or genetic drift (Wright 1931). The distribution of low frequency unique alleles detected across populations and the lack of evidence for a recent expansion event support the hypothesis that this lack of differentiation is a result of gene flow and not common ancestry. Our estimates of migration using Slatkin's $\hat{M}$ show a minimum of 2.9 migrants per generation between population pairs (Table 7), but gene flow can be variable and unpredictable among populations due to a wide array of demographic and environmental factors (Daly and Patton 1990) and estimates of absolute numbers of migrants are not reliable using microsatellite markers (Balloux and Lugon-Moulin 2002). Genetic variance among populations (F_{ST}) is only an indirect measure of gene flow and can be misleading when translated into dispersal rates (Whitlock and McCauley 1999). The estimate of gene flow ($Nm = 5.5$) using the conditional average frequency of private alleles is perhaps more accurate, but should not be the only means used to draw inference to population structure. Therefore, we also rely on the natural history of tortoises and our observation of inter-population movement to draw our conclusions. The most likely

scenario for the desert tortoise is that gene flow occurs not at a regular rate, but with varying frequencies over time.

Similar measures of gene flow, based on microsatellite data, were found in populations of geometric tortoise (*Psammobates geometricus*) in the western Cape Province of South Africa (F_{ST} = 0.031; Cunningham et al. 2002). This species shares a similar natural history with the desert tortoise in that the landscape contains physical barriers, such as mountains, that separate populations. In addition, the species is also long-lived and is faced with extreme habitat fragmentation due to human development.

Movement Barriers

The modern landscape of southern Arizona contains many recently constructed anthropogenic barriers that hinder or prevent movements of tortoises between populations, and, hence, disturb historic patterns of gene flow (Figures 5 and 6). During emigration of radiotelemetered tortoise RK459 from the Rincon Mountains to the Santa Rita Mountains, researchers facilitated her movement across several anthropogenic barriers, such as fence lines, railroad tracks, the interstate (I-10), and interactions with the public (Appendix D). This demonstrates that desert tortoises are capable and sometimes motivated to disperse great distances. The genetic data confirm that these movements result in the exchange of genetic material among adjacent populations. Our estimates of migration rates are per generation, and since tortoises exhibit extremely long generation times with respect to the recent proliferation of landscape barriers, the gene flow we observed predates habitat fragmentation and should not be taken as evidence that immigration still occurs. What is exemplified by tortoise RK459 is that urban topography in our modern landscape makes such movements by tortoises virtually impossible without assistance.

Population Viability

Because Sonoran desert tortoise effective population sizes are likely small, dispersal events probably play an important role in the long-term maintenance of populations. Life history traits of the desert tortoise, a long-lived species with delayed sexual maturity, suggest that there are severe constraints on the ability of populations to respond to chronic disturbances (Congdon et al. 1993). Demographic modeling and life tables for tortoises indicate that adult females are the most crucial life stage for population longevity, such that even a small increase in their mortality rate could result in a population crash (Berry et al. 1994, Doak et al. 1994). It is unlikely that a closed population of desert tortoises experiencing a dramatic reduction in adult survivorship would be able to offset that loss through compensatory increase in reproductive output. The high level of gene flow among populations suggests that if a population were to experience a catastrophic decline as a result of drought or other stochastic event, its recovery may rely heavily on the immigration of new individuals from adjacent mountain ranges to repopulate it.

MANAGEMENT AND RESEARCH IMPLICATIONS

Genes leave a trail of the movements of animals over time. The ultimate application of this genetic information is not necessarily how to maintain the genetic integrity of tortoise populations, but it gives us insight that tortoises historically dispersed and successfully reproduced in other populations. These movements may be critical to the persistence of desert tortoise populations. Because many historic dispersal routes are no longer available to desert tortoises as a result of anthropogenic landscape change (Figures 5 and 6), informed management strategies need to be in place to facilitate the long-term persistence of Sonoran desert tortoise populations.

An excellent example of a desert tortoise population imperiled by landscape change is the population at Tumamoc Hill within the city limits of Tucson. Tumamoc Hill hosts a small (<30), but currently healthy population of tortoises. This population is essentially an island completely surrounded by urban development. The effects are apparent in the tortoise population; the population is small and many of the individuals we found exhibited shell trauma from domestic or feral dog attacks (Appendix A). The proximity of this site to people's homes also makes the tortoise population vulnerable to escaped or released domestic tortoises that are a potential source of disease to wild populations (AIDTT 1996). The probability of this population experiencing a decline from human-related activities seems inevitable. Since there is no dependable way for new tortoises to naturally immigrate into the population due to the proliferation of heavily traveled roads surrounding it, the population will likely be extirpated. Because of the long lifespan of desert tortoises, a local extinction could take decades.

We encourage the application of genetic information directly to management and research in the following areas:

Designation of Management Units

Knowledge of the genetic variation among populations in a species can be used to define evolutionary significant units (ESU's) and management units (MU's). For example, Hedrick et al. (2001) used molecular techniques to differentiate populations of Gila topminnow (*Poeciliopsis o. occidentalis*) from four major watersheds in Arizona to aid in the management and conservation of this endangered species. Now that informative microsatellite markers have been identified for Sonoran populations of the desert tortoise, scoring new individuals for genetic variability is relatively straightforward and inexpensive. We encourage further molecular research of tortoises from other parts of their range in Arizona. It may be possible to collect genetic information from scat or carcasses, which would eliminate the need to conduct invasive procedures on tortoises in the field and would allow researchers and resource managers to easily collect samples during subsequent research and monitoring. It is likely that analysis of population genetic structure throughout the range of tortoises in Arizona will identify distinctive, regional characteristics. This information could facilitate the identification and management of ESU's, (e.g., Kingman District, Arizona Strip, Western Arizona, Southern Arizona, etc.). The Arizona Game and Fish Department and other resource managers could use this information to design conservation strategies specific to each unique region.

Identification of Domestic Tortoises

Desert tortoises are popular pets in southern Arizona, and as urban boundaries encroach further upon wild areas there is greater potential for interaction between domesticated and wild desert tortoises. Disease spread by the release or escape of captive desert tortoises is considered a major threat to wild populations (AIDTT 1996). In addition, concern has been raised that admixture with more distantly related individuals could cause genetic contamination of local populations. One of the tortoises genotyped in this study was found on an AGFD monitoring plot in a remote part of the West Silverbell Mountains with red, white, and green paint on its carapace, (Appendix A; WSB84). The tortoise was first found by P. Woodman in 1995 with a full Christmas scene painted on the shell and had therefore been living successfully in the area for at least 5 years. It is unknown if the tortoise was found and painted at this location or was a captive that was released or escaped. Our analysis revealed that this tortoise did not differ genetically from others in the region or the West Silverbell Mountains population.

Molecular techniques can be used to identify the population origin of individuals and have been proposed for detecting wildlife poaching (Manel et al. 2002), but the capability of identifying individuals could have a variety of applications. Microsatellite markers allow us to "fingerprint" individuals and could potentially be used to help confirm the identity of escaped or released captive tortoises in areas to which they are not indigenous. Since we detected very little differentiation among populations, we do not see this as a feasible within the limited geographic region of this study. However, if a larger area were studied and additional microsatellite markers were used, genotypes might be assignable to particular areas. It is already known that individuals from the Mojave Desert population are genetically distinct from those in the Sonoran Desert based on allozyme and mtDNA data (Lamb et al. 1989, Lamb and Lydeard 1994, Ostentoski and Lamb 1995, McLuckie et al. 1999).

Landscape Connectivity

The genetic data indicate that in many cases it is imperative for the long-term viability of tortoise populations that individuals are able to move between adjacent populations. Assessing what constitutes a barrier to movement for tortoises will better facilitate human development in areas that still maintain connectivity between populations. While a roadway may not be a barrier to a large ungulate, it may be impenetrable to a tortoise. Tortoises are able to cross some barriers and have been shown to use culverts (Ruby et al. 1994). Fencing or concrete barriers along highways may also help guide tortoises toward appropriate crossing areas and prevent roadkill. Placement of culverts or corridors needs to be specifically for tortoises, as corridors designed for general wildlife use may not be effective (Barrett et al. 1990). While long-distance movement has been documented for adult tortoises (this study; Barrett 1990, Barrett et al. 1990, Averill-Murray and Klug 2000), dispersal information of juveniles is severely lacking. Understanding the movement patterns for these early life stages is critical for long-term management of tortoise populations.

Translocation

Molecular data can be used to establish effective translocation strategies for wildlife. For example, Maudet et al. (2002) evaluated translocation strategies for the threatened alpine ibex (*Capra ibex*) using microsatellite markers. Microsatellites proved valuable for assessing the rate of gene flow, the level of divergence between populations, the genetic variability of the populations, and the number of individuals for translocation.

Translocation of tortoises from nearest-neighbor populations may become necessary to recover or maintain small populations isolated by anthropogenic barriers. Tortoises generally exhibit strong site tenacity (Barrett et al. 1990, Bailey 1992), and translocation studies of reptiles indicate that they generally fare poorly in unfamiliar areas (Barrett et al. 1990, Dodd and Seigel 1991, Reinert and Rupert 1999). However, recent studies in the Mojave Desert indicate that translocation can be used as a conservation tool for the desert tortoise (Nussear et al. 2000) and that translocation may be an effective strategy for supplementing depauperate populations of desert tortoise (Tracy et al. 2000). Currently in Arizona, tortoises are sometimes relocated short distances to adjacent areas during construction projects (AIDTT 1996).

Before tortoise inter-population translocation strategies are implemented in the Sonoran Desert, effects of translocation on the survivorship of relocated individuals and the populations into which they are introduced need to be evaluated and the potential for disease transmission from one population to another needs to be assessed. While it may be tempting to apply the OMPG rule to isolated tortoise populations not experiencing a decline, different schedules of supplementation may be appropriate dependent on environmental and demographic conditions specific to each population (Mills and Allendorf 1996). Population viability analysis would help determine if regular translocation is necessary as a maintenance strategy and the appropriate amount of gene flow needed to maintain the genetic integrity of Sonoran desert tortoise populations.

Population Viability Analysis

Population viability analysis (PVA) has become a commonly used tool in addressing issues of extinction and loss of genetic diversity in small and often fragmented populations of threatened species (Gilpin and Soule 1986, Clark et al. 1990, Lindenmayer et al. 1993, Hedrick et al. 1996). PVAs are computer models that systematically evaluate the relative importance of factors that may place a population at risk (Soulé 1987). PVAs cannot estimate exact extinction probabilities, but attempt to identify the various importance of factors being considered and evaluate various management strategies (Clark et al. 1990). Now, with information on tortoise gene flow provided by this report and effective methods for measuring population densities (distance sampling; Swann et al. 2002), a complete PVA would be a valuable tool for assisting managers in maintaining tortoise populations in a fragmented landscape.

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APPENDIX A. Desert tortoise field capture data

Table A1. Location and capture data for tortoises in southern Arizona from which genetic samples were collected.

Date	Location	Tortoise ID	Sex	MCL (mm)	notes:
4-Aug-00	Desert Peak	DP100	M	212	epoxyed number only; did not notch
4-Aug-00	Desert Peak	DP101	M	232	epoxyed number only; did not notch
4-Aug-00	Desert Peak	DP102	F	222	epoxyed number only; did not notch
19-Aug-00	Desert Peak	DP103	M	220	epoxyed number only; did not notch
19-Aug-00	Desert Peak	DP104	M	240	epoxyed number only; did not notch
19-Aug-00	Desert Peak	DP105	M	249	epoxyed number only; did not notch
11-Aug-01	Desert Peak	DP106	F	184	epoxied number only
11-Aug-01	Desert Peak	DP107	M	235	epoxied number only
19-Aug-00	Desert Peak	DP32	F	206	marked from previous study; Mercy Vaughan
19-Aug-00	Desert Peak	DP38	F	228	marked from previous study; Mercy Vaughan
19-Aug-00	Desert Peak	DP720	F	214	marked from previous study; Mercy Vaughan
19-Aug-00	Desert Peak	DP99	F	235	marked from previous study; Mercy Vaughan
11-Oct-00	Florence	FL400		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL403		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL404		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL405		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL406		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL409		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL410		0	AGFD Study Plot; R. Averill-Murray
11-Oct-00	Florence	FL502		0	AGFD Study Plot; R. Averill-Murray
6-May-01	Mother's Day Fire	MDB000	M	0	Collected during AGFD repro/disease study
6-May-01	Mother's Day Fire	MDB106	M	0	Collected during AGFD repro/disease study
6-May-01	Mother's Day Fire	MDB339	M	0	Same as Tortoise #781. "Harry"
6-May-01	Mother's Day Fire	MDB410	F	242	Collected during AGFD repro/disease study
14-Jul-00	Mother's Day Fire	MDB410	F	236	radio-telemetered tortoise
14-Jul-00	Mother's Day Fire	MDB441	M	224	not radioed
15-Jul-00	Mother's Day Fire	MDB483	F		radio-telemetered tortoise (freq. 166.974)
6-May-01	Mother's Day Fire	MDB483	F	228	Collected during AGFD repro/disease study
15-Jul-00	Mother's Day Fire	MDB712	M		radio-telemetered tortoise (freq. 166.433)
14-Jul-00	Mother's Day Fire	MDB721	M		radio-telemetered tortoise
14-Jul-00	Mother's Day Fire	MDB781	M	264	radio-telemetered tortoise (freq. 166.781)
14-Jul-00	Mother's Day Fire	MDB827	M		Tortoise #000, "Edgar"
15-Jul-00	Mother's Day Fire	MDB876	F		Tortoise #143, "Chloe"
14-Jul-00	Mother's Day Fire	MDB928	M	254	Tortoise #722 "Rocky"
12-Aug-00	Picacho Mountains	PM1	F	247	epoxyed number only; did not notch
4-Aug-01	Picacho Mountains	PM10	F	240	previously radioed
23-Sep-00	Picacho Mountains	PM107	F	238	marked on this date

Date	Location	Tortoise ID	Sex	MCL (mm)	notes:
23-Sep-00	Picacho Mountains	PM108	F	239	marked on this date
4-Aug-01	Picacho Mountains	PM109	F	262	new capture, notched
4-Aug-01	Picacho Mountains	PM111	M	269	new capture, notched
4-Aug-01	Picacho Mountains	PM112	M	244	new capture, notched
12-Aug-00	Picacho Mountains	PM2	M	213	epoxyed number only; did not notch
23-Sep-00	Picacho Mountains	PM28	M	263	marked; S. Barrett, transmitter still attached
12-Aug-00	Picacho Mountains	PM3	F	249	epoxyed number only; did not notch
12-Aug-00	Picacho Mountains	PM4	M	290	epoxyed number only; did not notch
12-Aug-00	Picacho Mountains	PM5	M	274	epoxyed number only; did not notch
23-Sep-00	Picacho Mountains	PM55	F	226	marked from previous study; Sherry Barrett
23-Sep-00	Picacho Mountains	PM59	M	233	marked from previous study; Sherry Barrett
12-Aug-00	Picacho Mountains	PM6	M	271	epoxyed number only; did not notch
23-Sep-01	Picacho Mountains	PM7	M	240	epoxied number only; south of previous PM location
23-Sep-01	Picacho Mountains	PM8	F	276	epoxied number only; south of previous PM location
23-Sep-00	Picacho Mountains	PM96	F	242	marked from previous study; Sherry Barrett
15-Sep-01	Rocking K	RK103		0	collected during AGFD repro/disease study
15-Sep-01	Rocking K	RK123		0	collected during AGFD repro/disease study
15-Sep-01	Rocking K	RK143		0	collected during AGFD repro/disease study
15-Sep-01	Rocking K	RK153		0	collected during AGFD repro/disease study
31-Aug-01	Rocking K	RK404	M	0	Radioed tortoise
17-Aug-00	Rocking K	RK411	M	222	radio-telemetered
15-Sep-01	Rocking K	RK412		0	collected during AGFD repro/disease study
23-Aug-00	Rocking K	RK413	F	249	radio-telemetered
5-May-01	Rocking K	RK414	M	0	Collected during AGFD repro/disease study
23-Aug-00	Rocking K	RK416	F	227	radio-telemetered
5-May-01	Rocking K	RK422	F	0	Collected during AGFD repro/disease study
15-Jul-01	Rocking K	RK429	F	0	During BBC Filming
15-Jul-01	Rocking K	RK435	M	0	During BBC Filming
17-Aug-00	Rocking K	RK459	F	240	radio-telemetered
15-Sep-01	Rocking K	RK468		0	collected during AGFD repro/disease study
17-Aug-00	Rocking K	RK479	F	226	radio-telemetered
5-May-01	Rocking K	RK479	F	0	Collected during AGFD repro/disease study
5-May-01	Rocking K	RK480	F	0	Collected during AGFD repro/disease study
23-Aug-00	Rocking K	RK480	F	258	radio-telemetered
9-Aug-00	Rocking K	RK481	M	257	radio-telemetered
5-May-01	Rocking K	RK481	M	0	Collected during AGFD repro/disease study
9-Aug-00	Rocking K	RK482	M	262	radio-telemetered
5-May-01	Rocking K	RK485	F	0	Collected during AGFD repro/disease study
9-Aug-00	Rocking K	RK485	F	236	radio-telemetered
5-May-01	Rocking K	RK486	F	0	Collected during AGFD repro/disease study
17-Aug-00	Rocking K	RK486	F	234	radio-telemetered
5-May-01	Rocking K	RK503		0	Collected during AGFD repro/disease study
9-Aug-00	Rocking K	RK510	F	280	radio-telemetered

Date	Location	Tortoise ID	Sex	MCL (mm)	notes:
5-May-01	Rocking K	RK510	F	0	Collected during AGFD repro/disease study
31-Aug-01	Rocking K	RK511	M	0	radioed tortoise
15-Sep-01	Rocking K	RK514		0	collected during AGFD repro/disease study
15-Sep-01	Rocking K	RK515		0	collected during AGFD repro/disease study
5-May-01	Rocking K	RK530	M	0	Collected during AGFD repro/disease study
5-May-01	Rocking K	RK532	M	0	Collected during AGFD repro/disease study
5-May-01	Rocking K	RK564		0	Collected during AGFD repro/disease study
19-Sep-01	Ragged Top	RT125		0	collected during disease study / distance sampling
22-Jul-00	Ragged Top	RT144	unk.	106	
22-Jul-00	Ragged Top	RT145	F	218	
22-Jul-00	Ragged Top	RT146	unk.	104	
22-Jul-00	Ragged Top	RT147	M	251	
19-Sep-01	Ragged Top	RT148		0	
22-Jul-00	Ragged Top	RT148	F	231	nematodes in urine
22-Jul-00	Ragged Top	RT149	F	204	
22-Jul-00	Ragged Top	RT150	unk.	161	blood engorged sand flies
11-Oct-00	Ragged Top	RT400	M	213	marked on this date
11-Oct-00	Ragged Top	RT401	F	214	marked on this date
30-Jun-01	Ragged Top	RT402	F	230	Radio attachment @ RT
19-Sep-01	Ragged Top	RT402		0	
19-Sep-01	Ragged Top	RT403		0	
30-Jun-01	Ragged Top	RT403	unk.	186	Radio attachment @ RT
19-Sep-01	Ragged Top	RT408		0	
1-Jul-01	Ragged Top	RT409	F	215	Radio attachment @ RT
19-Sep-01	Ragged Top	RT409		0	
1-Jul-01	Ragged Top	RT410	unk.	177	Radio attachment @ RT
1-Jul-01	Ragged Top	RT411	unk.	142	Radio attachment @ RT
19-Sep-01	Ragged Top	RT413		0	
19-Sep-01	Ragged Top	RT417		0	
19-Sep-01	Ragged Top	RT421		0	
13-Aug-01	Ragged Top	RT423	F	203	AGFD distance sampling; radioed tortoise
13-Aug-01	Ragged Top	RT427	F	208	AGFD distance sampling
13-Aug-01	Ragged Top	RT428	F	226	AGFD distance sampling
10-Oct-00	Sugarloaf Mountain	SL1		0	AGFD Study Plot
10-Oct-00	Sugarloaf Mountain	SL14		0	AGFD Study Plot
10-Oct-00	Sugarloaf Mountain	SL17		0	AGFD Study Plot
10-Oct-00	Sugarloaf Mountain	SL29		0	AGFD Study Plot
13-Sep-00	Sugarloaf Mountain	SL3	F		Radio-telemetered tortoise; AGFD
13-Sep-00	Sugarloaf Mountain	SL318	M		tortoise not radioed; AGFD
13-Sep-00	Sugarloaf Mountain	SL43	M	230	tortoise not radioed; AGFD shelter 128
13-Sep-00	Sugarloaf Mountain	SL45	F		Radio-telemetered tortoise; AGFD
13-Sep-00	Sugarloaf Mountain	SL46	F		Radio-telemetered tortoise; AGFD shelter 313
13-Sep-00	Sugarloaf Mountain	SL56	F		Radio-telemetered tortoise; AGFD

Date	Location	Tortoise ID	Sex	MCL (mm)	notes:
13-Sep-00	Sugarloaf Mountain	SL57	F		Radio-telemetered tortoise; AGFD
10-Oct-00	Sugarloaf Mountain	SL58		0	AGFD Study Plot
13-Sep-00	Sugarloaf Mountain	SL604	M	255	tortoise not radioed; AGFD shelter 128
13-Sep-00	Sugarloaf Mountain	SL63	F		Radio-telemetered tortoise; AGFD shelter 128
13-Sep-00	Sugarloaf Mountain	SL65	F		Radio-telemetered tortoise; AGFD
13-Sep-00	Sugarloaf Mountain	SL66	F		Radio-telemetered tortoise; AGFD shelter 430
13-Sep-00	Sugarloaf Mountain	SL67	F		Radio-telemetered tortoise; AGFD
13-Sep-00	Sugarloaf Mountain	SL68	F		Radio-telemetered tortoise; AGFD shelter 346
13-Sep-00	Sugarloaf Mountain	SL69	F		Radio-telemetered tortoise; AGFD
13-Sep-00	Sugarloaf Mountain	SL72	F		Radio-telemetered tortoise; AGFD
13-Sep-00	Sugarloaf Mountain	SL73	F		Radio-telemetered tortoise; AGFD shelter 316
13-Sep-00	Sugarloaf Mountain	SL80	F		Radio-telemetered tortoise; AGFD shelter 371
13-Sep-00	Sugarloaf Mountain	SL81	F		Radio-telemetered tortoise; AGFD
10-Oct-00	Sugarloaf Mountain	SL86		0	AGFD Study Plot
4-May-01	Sugarloaf Mountain	SL9	M	0	No Data
10-Oct-00	Sugarloaf Mountain	SL94		0	AGFD Study Plot
9-Sep-00	Tumamoc Hill	TH01	F	201	epoxyed number only. Shell damage from dog attack
9-Sep-00	Tumamoc Hill	TH02	M	234	epoxyed number only. Shell damage from dog attack
9-Sep-00	Tumamoc Hill	TH04	F	235	epoxyed number only. Deformed shell
9-Sep-00	Tumamoc Hill	TH05	M	244	epoxyed number only. Shell damage from dog attack
21-Sep-00	Tumamoc Hill	TH06	M	242	epoxyed number only. Shell damage from dog attack
28-Sep-00	Tumamoc Hill	TH07	M	258	epoxyed number only; did not notch
28-Sep-00	Tumamoc Hill	TH08	F	231	epoxyed number only. Shell damage from dog attack
21-Sep-01	Tumamoc Hill	TH11	F	224	epoxyed number only; did not notch
28-Sep-01	Tumamoc Hill	TH12	F	211	epoxyed number only; did not notch
26-Aug-00	Tucson Mountains	TM1	unk.	167	tortoise left unmarked
16-Sep-00	Tucson Mountains	TM10	M	213	marked from previous study
26-Aug-00	Tucson Mountains	TM180	unk.	120	marked from previous study
26-Aug-00	Tucson Mountains	TM182	F	195	marked from previous study
18-Aug-01	Tucson Mountains	TM183	F	244	marked on this date
26-Aug-00	Tucson Mountains	TM2	F	192	tortoise left unmarked
16-Sep-00	Tucson Mountains	TM21	F	254	marked from previous study
16-Sep-00	Tucson Mountains	TM223	F	238	marked on this date
26-Aug-00	Tucson Mountains	TM3	F	248	tortoise left unmarked
26-Aug-00	Tucson Mountains	TM4	F	242	tortoise left unmarked
1-Sep-01	Tucson Mountains	TM431	F	225	Marked on this date
1-Sep-01	Tucson Mountains	TM711	F	238	previously marked
26-Aug-00	Tucson Mountains	TM712	F	228	marked from previous study
26-Aug-00	Tucson Mountains	TM788	F	213	marked from previous study
18-Aug-01	Tucson Mountains	TM792	F	233	previously marked, repaired shell?
18-Aug-01	Tucson Mountains	TM803	M	222	previously marked
18-Aug-01	Tucson Mountains	TM811	F	256	marked from previous study
26-Aug-00	Tucson Mountains	TM9	M	272	marked from previous study

Date	Location	Tortoise ID	Sex	MCL (mm)	notes:
4-Oct-00	West Silver Bells	WSB1	F	243	marked; AGFD study plot
4-Oct-00	West Silver Bells	WSB11	M	267	marked; AGFD study plot
7-Aug-01	West Silver Bells	WSB114	F	235	AGFD distance sampling; recapture
4-Oct-00	West Silver Bells	WSB116	M	257	marked; AGFD study plot
7-Aug-01	West Silver Bells	WSB146	F	238	AGFD distance sampling; recapture
3-Oct-00	West Silver Bells	WSB16	F	215	marked; AGFD study plot
6-Aug-01	West Silver Bells	WSB161	M	230	AGFD distance sampling; recapture
4-Oct-00	West Silver Bells	WSB176	F	204	marked; AGFD study plot
7-Aug-01	West Silver Bells	WSB180	M	261	AGFD distance sampling; new capture
7-Aug-01	West Silver Bells	WSB181	F	252	AGFD distance sampling; new capture
4-Oct-00	West Silver Bells	WSB34	F	253	marked; AGFD study plot
3-Oct-00	West Silver Bells	WSB37	M	274	marked; AGFD study plot
4-Oct-00	West Silver Bells	WSB44	F	244	marked; AGFD study plot
4-Oct-00	West Silver Bells	WSB45	M	236	marked; AGFD study plot
4-Oct-00	West Silver Bells	WSB50	M	260	marked; AGFD study plot
4-Oct-00	West Silver Bells	WSB57	F	239	marked; AGFD study plot
7-Aug-01	West Silver Bells	WSB75	F	230	AGFD distance sampling; recapture
4-Oct-00	West Silver Bells	WSB77	F	249	marked; AGFD study plot
4-Oct-00	West Silver Bells	WSB84	M	247	AGFD study plot: paint on shell, captive? Originally found by P. Woodman in 1995.

APPENDIX B. Home range size for desert tortoises at Saguaro National Park, Rincon Mountain District

Table B1. Radiotelemetry data for thirty four tortoises from Saguaro National Park, Rincon Mountain District. Average minimum convex polygon home range size (MCP) was not significantly different between the Mother's Day Fire and the Rocking K sites after accounting for sex, size (MCL; midline carapace length), and number of location points collected for each tortoise ($F_{4,33} = 0.425$, $P>0.789$).

Site	Tort #	MCL (MM)	# Locations	Sex	MCP (ha)
Mother's Day Fire	MDB000	253	69	Male	4.61
Mother's Day Fire	MDB106	273	51	Male	16.02
Mother's Day Fire	MDB339	264	67	Male	2.81
Mother's Day Fire	MDB410	242	134	Female	22.6
Mother's Day Fire	MDB483	228	156	Female	10.9
Mother's Day Fire	MDB712	225	153	Male	19.6
Mother's Day Fire	MDB721	217	133	Male	6.88
Mother's Day Fire	MDB876	227	62	Female	35.6
Mother's Day Fire	MDB928	254	69	Male	29.94
Rocking K	RK103	231	15	Female	6.72
Rocking K	RK404	267	112	Male	14.29
Rocking K	RK411	222	68	Male	81.58
Rocking K	RK412	242	90	Male	4.75
Rocking K	RK413	249	43	Female	7.44
Rocking K	RK414	230	92	Male	9.86
Rocking K	RK416	227	42	Female	2.16
Rocking K	RK422	222	89	Female	6.41
Rocking K	RK429	242	77	Female	70.1
Rocking K	RK435	235	87	Male	24.38
Rocking K	*RK459	240	20	Female	10,692.2
Rocking K	RK468	220	24	Female	1.9
Rocking K	RK479	226	65	Female	21.65
Rocking K	RK480	249	67	Female	22
Rocking K	RK481	257	52	Male	30.56
Rocking K	RK482	262	51	Male	11.44
Rocking K	RK485	236	62	Female	6.73
Rocking K	RK486	234	64	Female	26.12
Rocking K	RK510	280	72	Female	58.04
Rocking K	RK511	253	60	Male	23.47
Rocking K	RK514	230	25	Female	0.334
Rocking K	RK515	245	31	Female	11.33
Rocking K	RK530	247	51	Male	5.34
Rocking K	RK532	267	49	Male	9.11
Rocking K	RK564	254	12	Female	0.33

(MEAN: 18.01, 95% C.I. 11.34 to 24.69 ha)

*Tortoise made long distance movement; not included in home range size analysis

APPENDIX C. Frequency and distribution of alleles across populations

Table C1. Diversity indices for seven microsatellite loci in nine populations of desert tortoise. # = number of individuals genotyped. Size = the range of allele repeat lengths. H_{obs} = observed heterozygosity and H_{exp} = expected heterozygosity. *Prob* = significance level and S.D. = standard deviation of randomization tests for Hardy-Weinberg equilibrium. F_{IS} = Weir and Cockerham's inbreeding estimator (1984).

Goag3

Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	6-8	0.3333	0.3696	1.000	<0.001	-0.114	1.000
Florence	8	6-8	0.6250	0.5750	1.000	<0.001	-0.296	1.000
Rincon Mountains (SNP)	38	6-8	0.4359	0.4612	0.761	0.004	0.056	0.757
Picacho Mountains	18	6-8	0.3333	0.4587	0.272	0.004	0.206	0.260
Ragged Top	22	6-8	0.3182	0.3541	0.376	0.004	0.104	0.384
Sugarloaf	27	6-8	0.2963	0.2998	1.000	<0.001	-0.106	1.000
Tumamoc Hill	9	6-8	0.3333	0.2941	1.000	<0.001	-0.143	1.000
Tucson Mountains	18	6-8	0.5556	0.4524	0.771	0.004	-0.236	0.755
West Silver Bell Mountains	18	6-8	0.1111	0.1619	1.000	<0.001	-0.015	1.000

Goag4

Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	10-23	0.2500	0.2355	1.000	<0.001	-0.065	1.000
Florence	8	10-15	0.6250	0.4583	0.487	0.005	-0.400	0.487
Rincon Mountains (SNP)	38	9-25	0.7692	0.7543	0.858	0.003	-0.020	0.861
Picacho Mountains	18	9-29	0.7778	0.7318	0.543	0.002	-0.065	0.558
Ragged Top	22	9-25	0.6818	0.7600	0.790	0.002	0.082	0.750
Sugarloaf	27	9-24	0.5185	0.5646	0.616	0.003	0.033	0.542
Tumamoc Hill	9	9-23	0.7778	0.7712	0.739	0.002	-0.009	0.702
Tucson Mountains	18	9-23	0.4444	0.4349	0.526	0.003	-0.023	0.571
West Silver Bell Mountains	18	7-24	0.7222	0.7794	0.308	0.001	0.043	0.225

Goag5

Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	9-34	1.0000	0.9348	0.894	0.001	-0.073	0.739
Florence	8	14-29	0.7500	0.8500	0.391	0.002	0.097	0.407
Rincon Mountains (SNP)	38	9-38	0.8205	0.9261	0.036	<0.001	0.115	0.001
Picacho Mountains	18	6-34	1.0000	0.9222	0.034	<0.001	-0.087	0.014
Ragged Top	22	9-35	0.9091	0.9345	0.761	0.001	0.028	0.909
Sugarloaf	27	9-27	0.8889	0.8595	0.068	0.001	-0.035	0.072
Tumamoc Hill	9	9-32	1.0000	0.8824	0.433	0.001	-0.143	0.422
Tucson Mountains	18	15-38	0.8889	0.9111	0.040	0.001	0.016	0.174
West Silver Bell Mountains	18	12-33	0.7778	0.9270	0.024	0.001	0.165	0.043

Table C1, continued

Goag6

Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	15-27	0.0833	0.6486	<0.001	<0.001	0.863	<0.001
Florence	8	15-25	0.3750	0.8250	0.130	0.004	0.506	0.115
Rincon Mountains (SNP)	38	15-26	0.1539	0.6936	<0.001	<0.001	0.773	<0.001
Picacho Mountains	18	15-29	0.4444	0.6810	0.066	0.001	0.322	0.079
Ragged Top	22	15-51	0.5000	0.7484	0.006	0.001	0.335	0.002
Sugarloaf	27	17-49	0.4444	0.7219	<0.001	<0.001	0.365	0.001
Tumamoc Hill	9	15-25	0.3333	0.6863	0.086	0.002	0.455	0.071
Tucson Mountains	18	15-25	0.3333	0.6825	<0.001	<0.001	0.485	<0.001
West Silver Bell Mountains	18	15-52	0.3889	0.6762	<0.001	<0.001	0.402	0.001

Goag7

Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	12-18	0.3333	0.5978	0.0378	0.002	0.385	0.029
Florence	8	14-22	0.5000	0.7333	0.241	0.009	0.253	0.262
Rincon Mountains (SNP)	38	12-22	0.3846	0.6201	<0.001	<0.001	0.376	0.002
Picacho Mountains	18	12-22	0.6111	0.6698	0.753	0.003	0.043	0.742
Ragged Top	22	12-19	0.5000	0.5867	0.378	0.004	0.151	0.326
Sugarloaf	27	14-22	0.6667	0.7505	0.206	0.004	0.097	0.200
Tumamoc Hill	9	12-18	0.2222	0.4706	0.366	0.004	0.439	0.341
Tucson Mountains	18	12-22	0.5000	0.6048	0.332	0.002	0.126	0.339
West Silver Bell Mountains	18	12-21	0.3889	0.7540	<0.001	<0.001	0.466	0.001

Goag32

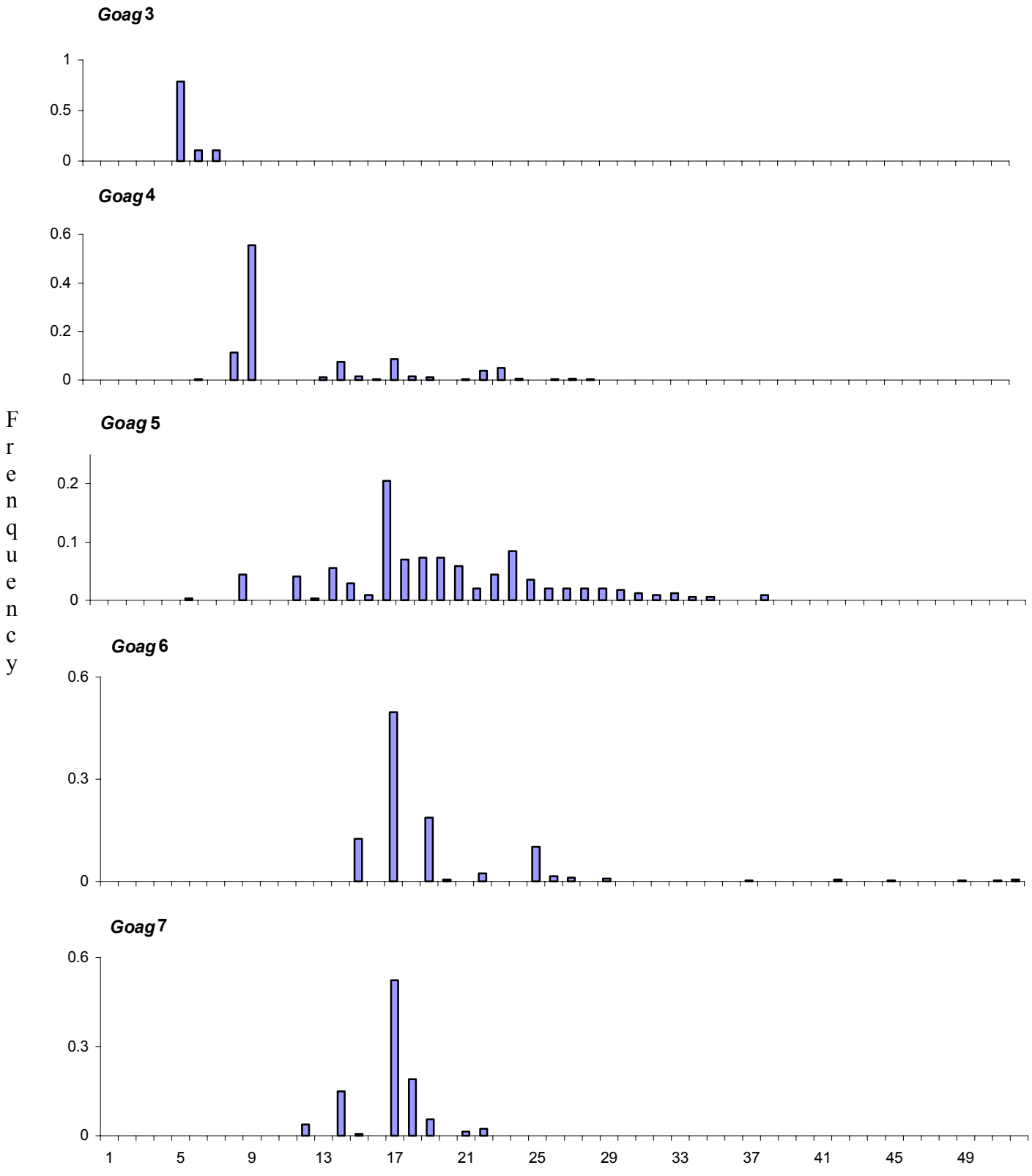
Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	5-6	0.2500	0.3007	1.000	<0.001	-0.100	1.000
Florence	8	5-6	0.1250	0.2417	1.000	<0.001	0.000	-
Rincon Mountains (SNP)	38	5-6	0.2051	0.2488	0.493	0.005	0.095	0.483
Picacho Mountains	18	5-6	0.1111	0.2032	0.177	0.005	0.460	0.169
Ragged Top	22	5-6	0.4091	0.4577	1.000	<0.001	0.041	1.000
Sugarloaf	27	5-6	0.4815	0.4004	0.288	0.005	-0.300	0.283
Tumamoc Hill	9	5-6	0.2222	0.3072	1.000	<0.001	-0.067	1.000
Tucson Mountains	18	5-6	0.5000	0.4365	1.000	<0.001	-0.150	1.000
West Silver Bell Mountains	18	5-6	0.1667	0.2079	1.000	<0.001	-0.062	1.000

Cm58

Population	#	Size	H_{obs}	H_{exp}	prob.	S.D.	F_{IS}	prob.
Desert Peak	12	12-13	0.3333	0.3587	1.000	<0.001	-0.158	1.000
Florence	8	12-13	0.3750	0.5917	0.539	0.005	0.300	0.530
Rincon Mountains (SNP)	38	12-13	0.3333	0.3353	1.000	<0.001	-0.060	1.000
Picacho Mountains	18	12-13	0.1667	0.3667	0.085	0.002	0.490	0.085
Ragged Top	22	12-13	0.3182	0.2738	1.000	<0.001	-0.167	1.000
Sugarloaf	27	12-13	0.3333	0.3599	1.000	<0.001	-0.009	1.000
Tumamoc Hill	9	12-13	0.1111	0.2157	1.000	<0.001	0.000	-
Tucson Mountains	18	12-13	0.1667	0.1571	1.000	<0.001	-0.062	1.000
West Silver Bell Mountains	18	12-13	0.1667	0.2079	1.000	<0.001	-0.062	1.000

Table C2. Distribution of unique and private alleles in nine populations of desert tortoise in southeastern Arizona.
T = total number of alleles from a population. U = number of alleles unique to the population, and the number of these that occur at a frequency >5% (private alleles) is indicated in parentheses. % = (U/T)x100. No allele unique to a population occurred at a frequency >7% in that population.

	Desert Peak			Florence			Picacho Mtns.			Ragged Top			Rincon Mtns.			Sugarloaf			Tucson Mtns.			Tumamoc Hill			West Silverbell Mtns.		
Locus	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%
*Goag*3	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	2	0	0	3	0	0
*Goag*4	4	0	0	3	0	0	10	3(1)	30	8	0	0	7	0	0	6	0	0	6	0	0	6	0	0	11	3	27
*Goag*5	14	0	0	8	0	0	14	1	7	20	1	5	21	0	0	11	0	0	13	0	0	10	0	0	14	0	0
*Goag*6	4	0	0	4	0	0	6	0	0	8	2	25	5	1(1)	20	6	2	33	6	1(1)	17	4	0	0	8	2(1)	25
*Goag*7	4	0	0	4	0	0	6	0	0	5	0	0	8	0	0	5	0	0	6	0	0	3	0	0	6	0	0
*Goag*32	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
Cm58	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
Total	33	0	0	26	0	0	43	4(1)	37	48	3	30	48	1(1)	40	35	2	33	38	1(1)	17	29	0	0	46	5(1)	52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49
Allele Size (number of repeats)

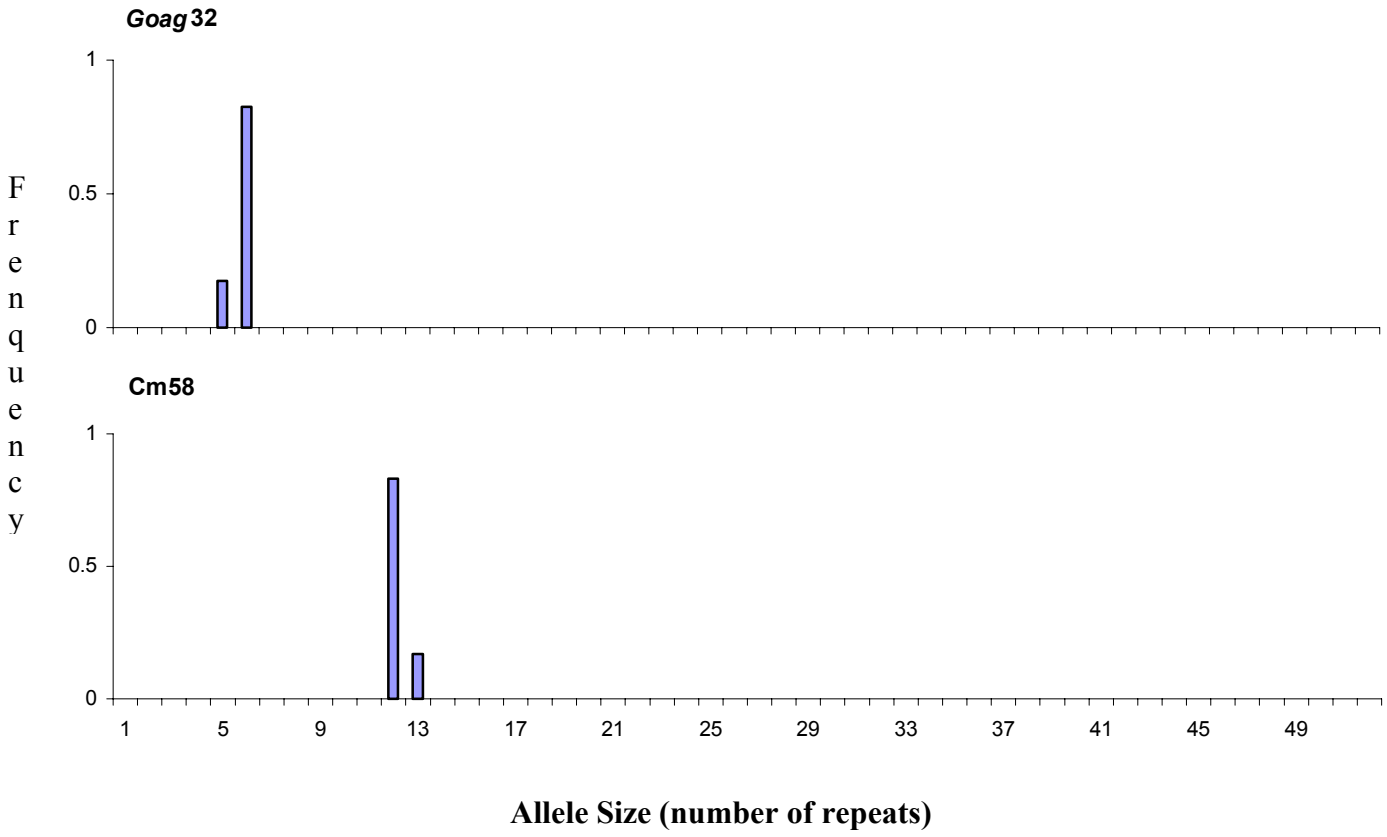

Figure C1. Allele distribution of 7 microsatellite loci in 9 populations of desert tortoise in Southern Arizona.

APPENDIX D. Long-distance Movement of a Radiotelemetered Tortoise

GOPHERUS AGASSIZII (Desert Tortoise): **MOVEMENT**. In the Sonoran Desert, *Gopherus agassizii* occurs in rocky foothills associated with saguaro cactus (*Carnegiea gigantea*) and foothill paloverde (*Parkinsonia microphylla*) characteristic of Upland Sonoran Desert Scrub plant community, (Brown 1992. Desert Plants 4:1-342). Although these populations appear to be isolated by low desert valleys, radiotelemetry data have shown that tortoises are capable of making long distance movements between populations (Barrett et al. 1990. final report, Bureau of Reclamation, Arizona Projects Office, Phoenix; Averill-Murray and Klug 2000. Technical Report 161, Arizona Game and Fish Dept., Phoenix). Long-distance movements between disjunct populations may facilitate genetic exchange (Britten et al. 1997. Copeia 1997:523-530) and be important for long-term maintenance of populations (T. Edwards in prep.). Here we report an extraordinary movement by a female *G. agassizii*, and the anthropogenic barriers encountered during this event. While desert tortoises are capable and sometimes motivated to make inter-population movements, the urban topography of our modern landscape makes such movements increasingly difficult.

We affixed a radio transmitter (AVM G3, AVM Industries, Colfax, CA) to an adult female *G. agassizii* (238 mm MCL, 2700 g) on 14 August 2000, as part of an ongoing study in the Rincon Mountains at Saguaro National Park (SNP), Tucson, Arizona. At the time of transmitter attachment, the tortoise exhibited symptoms of upper respiratory tract disease (URTD; nasal discharge, wheezing, occluded nares, exudate, etc.). We located the tortoise approximately every week, and by 6 September, she had moved roughly 500 m southwest of the original capture location, after which time we lost contact. On 18 September a SNP volunteer observed the tortoise along a roadway ca. 1.5 km south of the original locality. On 25 September, we found her approximately 8 km further south on a rocky slope surrounded by low-density housing. The terrain between these locations is primarily flat ground dominated by creosote (*Larrea divaricata*) and is atypical of Sonoran desert tortoise habitat (Barrett 1990. Herpetologica 46:202-206). On 02 October, we found her on private property along a chain-link fence. We obtained permission and put her across the fence. At this time, we affixed a note to the tortoise's carapace indicating she was part of a study at Saguaro National Park and included a contact phone number. We believe the tortoise over-wintered in Upland Sonoran Desert Scrub on a large expanse of private land; however, we did not receive a signal from her between 02 October 2000 and late July, 2001.

On 31 July 2001 we were contacted by a resident who had found her in Vail, Arizona, in the middle of the street at a railroad crossing (approximately 15 km south from where she was first marked). We placed her south of the railroad tracks, oriented in the same general direction she was moving but away from residential housing (within 0.5 km east of the crossing). Over the next two months, we received 3 phone calls from residents who had found the tortoise and brought her home. Each time, we returned her to uninhabited areas in the vicinity. During this period she remained within 1.5 km north of Interstate 10 (a 4 lane freeway due south of Vail), and traversed an approximately 3 km east-west distance. We made an *a priori* decision to facilitate the tortoise's movement across Interstate 10 if we observed continued southward movement.

On 29 August 2001, we located the tortoise on a frontage road beside I-10 and decided to transport her across the interstate. We placed the tortoise on a north-facing slope of the Santa Rita Mountains approximately 7 km south of the interstate where other tortoise sign was observed. We decided the 7 km distance was necessary because medium density housing and innumerable fences bisect land south of the interstate. The tortoise made several east-west movements along the foothill slopes at the new location, and on 18 September, 2001 we were contacted by a landowner who found her in the middle of a new residential development, 5 km west of the release point. We collected her and returned her to the original release site in the Santa Ritas. She over-wintered in the north end of the Santa Rita Mountains in the winter of 2001-02. We periodically tracked the tortoise to this same location until June 2002, at which point her transmitter failed prematurely. Not including the human facilitated movement of ca. 7 km, this tortoise moved more than 30 km straight line distance over the span of one year (figure D1).

On 22 August, 2002, we were contacted yet again by a family who found her on Interstate 10 under an overpass, 7 km north of her over-wintering site, toward the original capture site. We changed transmitters and re-released her at the first point of capture, at the south end of Saguaro National Park. The tortoise continues to periodically exhibit symptoms of URTDs.

This tortoise encountered several barriers that, without human facilitation, would likely have been insurmountable. A residential fence and an interstate highway both required human assistance to cross. We believe a set of railroad tracks may also have acted as a barrier and that the tortoise followed them for some distance before encountering a place to cross. Lastly, we note that at least 4 residents encountered the tortoise and contacted us. However, it is possible the tortoise would have become someone's illegal pet if the identifying label was not affixed to the carapace.

Any motive ascribed to this tortoise's movement would be purely speculative. However, it does demonstrate that desert tortoises are capable and sometimes motivated to make long-distance movements. Our genetic data confirm that these movements result in the exchange of genetic material among adjacent populations. This example demonstrates that inter-population movements by desert tortoises in our modern landscape may be virtually impossible without human intervention.

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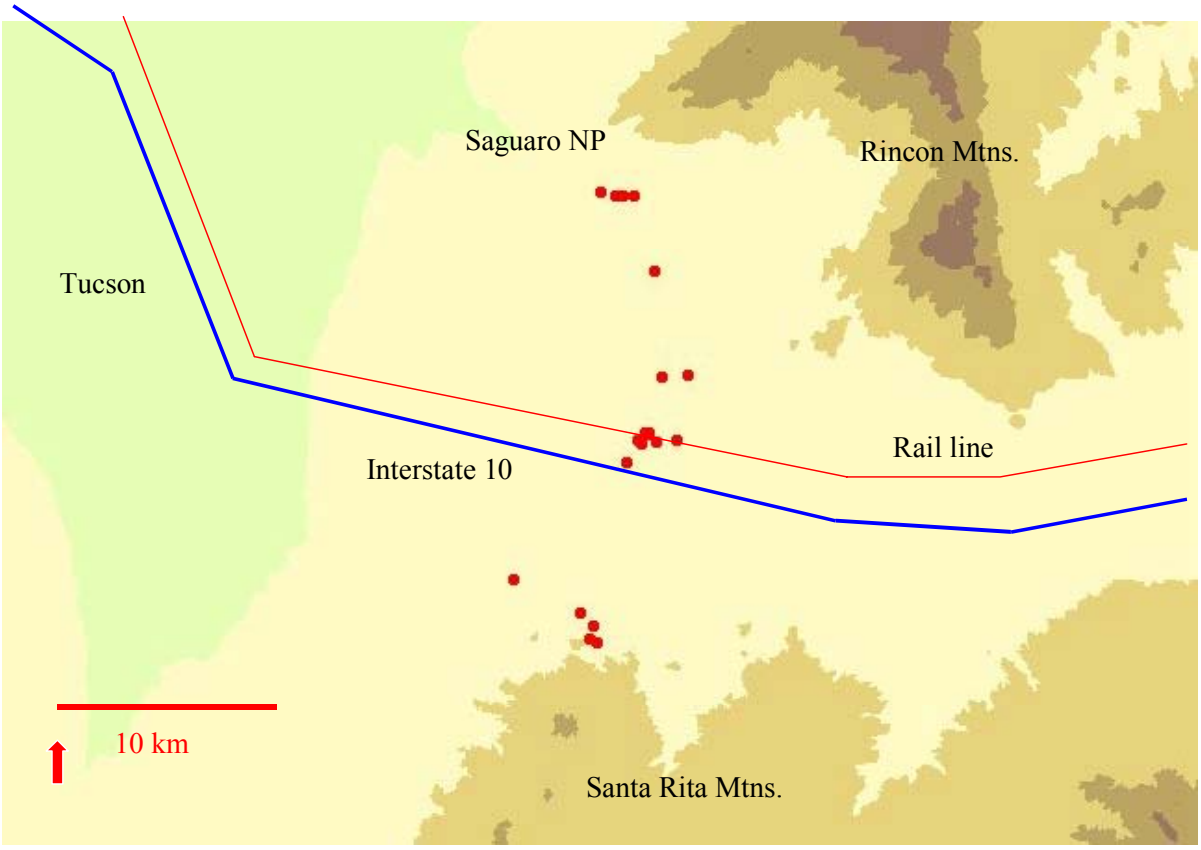

Figure D1. Long-distance movement made by tortoise RK459. (Locations of I-10 and rail line are approximate.